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OBSERVATIONS ON SOME EFFECTS OF COLOSTRUM DEPRIVATION IN THE CALF

By

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INTRODUCTION

The experiments recorded in this paper were a sequel to those of Blakemore, Davies, Eylenberg, Moore, Sellers and Worden (1948, 1954). They were carried out in order that the sequence of events resulting from the withholding of colostrum might be studied in some detail, and comparisons made with the development of calves that had received colostrum.

METHODS

General management. The calves were studied individually, the general management of all being as nearly as possible identical and resembling that adopted in the Cambridge experiments (Blakemore, *et al.*, 1948, 1954). Three only (22, 26 and 27) of the 34 animals observed were females.

The calves were collected from local farms, and were bled and weighed immediately on arrival. Those which were intended for the colostrum-deprived group were separated from their dams soon after birth. To ensure that these calves had not in fact suckled, a rapid test was carried out on their serum, 1 volume of which was diluted with 19 volumes of 18 per cent (w/v) Na_2SO_3, when an absence of any visible precipitate or turbidity was taken to indicate that absorption of colostrum globulin had not occurred. The calves which were to be used as controls were allowed to suckle naturally during the first day of life.

Each calf was allowed one gallon daily of bulk whole milk from the College Dairy Shorthorn herd. This was fed in equal amounts at 10 a.m. and at 5 p.m., the animals being taught to drink from a pail as soon as possible. The behaviour of the control calves suggested that pail-feeding was not a contributory factor in any of the disturbances noted among the colostrum-deprived group.

Clinical observations. These were, in the main, recorded at feeding times. Rectal temperatures were taken twice daily, and faeces samples and bodyweights were obtained prior to the morning feed. The observations on faeces and on gut contents were supplemented by study of material at the abattoir and in the field.

Haematological and biochemical data. The red and white cell pictures, and the serum and plasma protein levels, were followed throughout the experimental period in each case. Blood samples were taken before the morning feed by inserting a 1 mm. bore needle into the jugular vein and collecting directly into a bottle. When required, a 30 per cent (w/v)

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TABLE I

CLINICAL SUMMARY

Calf No.	Calf of Dam	Breed	Born	Age of Disposal	Scour			Other Symptoms and their duration (Days)	Weight at Birth (lb.)	Average daily Wt. Change (lb.)			
					First Observed	Duration	Other Days			1st wk.	2nd wk.	3rd wk.	4th wk.
				Calves deprived of colostrum									
13	2nd	Sh.	Jan.	D 2½	—	—	—	— —	65	—			
10	6th	Sh.	Jan.	D 3½	—	—	—	— —	87	—			
27	1st	Sh.	Jul.	D 10	45 hrs.	to 5 d.	8–12	— —	59·5	−0·5	—		
18	5th	Sh.	Mar.	S 16	—	—	—	Pyrexia 2, 9, 10	72	+0·8	+1·1	—	
14	10th	Sh.	Jan.	S 17	54 hrs.	to 12 d.	—	— —	78	+0·2	+1·0	—	
11	3rd	Sh.	Jan.	S 22	5 d.	to 6 d.	12–16	— —	75·5	+1·1	(	+0·6)	—
12	3rd	Sh.	Jan.	K 22	54 hrs.	to 11 d.	17–22	Arthritis 12–19	66·5	−1·1	(	−0·1)	
17	4th	Sh.	Feb.	S 28	48 hrs.	to 4 d.	20	Pyrexia 12, 13	82	−0·3	+1·4	(	+1·2)
16	5th	Sh.	Feb.	K 41	35 hrs.	to 7 d.	12–15	Arthritis 12 on.	64·5	−0·6	+0·9	+0·4	—
21	5th	Sh.	Apr.	S 44	—	—	12–15	Omphalitis 7–25	77·5	+0·6	+0·6	+0·5	+1·2
6	3rd	Sh.	Jan.	K 119	—	—	—	Arthritis 8 on.	79·5	+0·9	+1·0	(	+0·9)
19	3rd	Sh.	Apr.	K 91	48 hrs.	to 6 d.	20–25	Arthritis 9 on.	79	0	+0·9	+0·9	+0·2
30	2nd	F	Oct.	D 11	60 hrs.	to 6 d.	—	— —	73	−0·3			
36	5th	F	Mar.	S 25	5½ d.	to 6 d.	—	— —	96	(	+0·5)		
9	2nd	F	Nov.	S 27	6 d.	to 8 d.	—		82·5	+0·9	+0·6	+1·5	+1·6
29	8th	F	Oct.	* 30	30 hrs.	to 4 d.	—	Arthritis 17–26	86	+0·7	+0·9	+0·7	+1·3
32	9th	F	Oct.	S 56	48 hrs.	to 6 d.	—	— —	104	0	+0·1	+1·2	+1·1
31	3rd.	F	Oct.	K 59	72 hrs.	to 5 d.	—	Arthritis 13 on.	88	+0·5	+1·1	+0·4	+1·7
34	7th	F	Feb.	S 68	—	—	—	— —	104	+0·4	+1·1	+0·5	+1·0
28	1st	F	Mar.	S 82	—	—	—	— —	89·5	+1·1	+1·2	+1·6	+1·1
22	1st	X	Apr.	K 13	38 hrs.	to 13 d.	—	— —	65	−0·7	+0·2	—	
15	3rd	WB	Feb.	D 17	33 hrs.	to 2 d.	4, 7, 11–17	Omphalitis 2 on.	85	−1·1	−0·9	—	
40	2nd	X	Jun.	S 17	4 d.	to 9 d.	—	— —	78	+0·1	+1·0	—	
4	2nd	X	Mar.	S 75	—	—	—	— —	62	—	—	—	

TABLE 1—*Continued.*

Calf No.	*Calf of Dam*	*Breed*	*Born*	*Age of Disposal*	*Scour*			*Other Symptoms and their Duration (Days)*		*Weight at Birth (lb.)*	*Average daily Wt. Change (lb.)*			
					First Observed	*Duration*	*Other Days*				*1st wk.*	*2nd wk.*	*3rd wk.*	*4th wk.*
Calves receiving colostrum														
8	8th	X	Oct.	S 12	—	—	—	—	—	92·5	(	+1·4)	—	
7	2nd	F	Jul.	S 13	—	—	—	—	—	78·5	(	+1·3)	—	
39	2nd	Sh.	May	S 20	—	—	—	—	—	79	+0·67	(	+0·9)	—
26	1st	Sh.	Jul.	S 21	—	—	—	—	—	56·5	+1·8	+0·5	+0·8	—
37	3rd	Sh.	May	S 24	—	—	—	—	—	78	+0·3	(	+0·7)	—
38	1st	Sh.	May	S 24	—	—	—	—	—	74	+1·0	(	+0·9)	—
35	2nd	F	Mar.	S 31	—	—	—	—	—		+0·7	+0·7	+1·0	+0·8
20	3rd	Sh.	Aprl	S 45	—	—	—	—	—	62	+0·7	+1·2	+1·4	+1·4
5	1st	X	May	S 62	—	—	—	—	—	63·5	+0·3	(	+1·2)	—
33	3rd	F	Jan.	S 73	—	—	—	—	—	81	+0·7	+0·7	+0·6	+1·4

Sh. = Shorthorn. D = Dead.
F = Friesian. K = Killed.
X = Crossbred. S = Sold.
WB = Welsh Black. * = Died following vaccination.

Brackets indicate mean daily gain over a fortnight.

solution of potassium oxalate was employed as the anticoagulant, at a concentration of approximately 2 mg./ml. The blood cell counts were made by standard procedures using Thoma pipettes and Neubauer slides. Haemoglobin was estimated visually by Sahli's method. The packed cell volumes (P.C.V.) were measured in Wintrobe haematocrit tubes, centrifuged at 3,000 r.p.m. for 45 minutes in a centrifuge of effective radius 20 c. The readings recorded are those observed, and are uncorrected for entrapped plasma or for the effect of the added potassium oxalate. Leishmann's stain was used for the blood smears and, for the differential count, 300 to 400 cells were classified by methods similar to those described by Holman (1950). In the figures recorded here, the white cells are divided into the two main series also, the polymorphonuclear and the mononuclear. The plasma and serum proteins (N x 6.25) were estimated by a micro-Kjeldahl technique similar to that described by King (1946), but the ammonia was distilled into 2 per cent (w/v) boric acid prior to titration with 0.04 N-H_2SO_4. The methods for globulins were based on Campbell and Hanna's (1937) modification of Howe's (1921) method, using Na_2SO_3 instead of Na_2SO_4 for salting out. Precautions were taken to minimise the effects of albumin absorption by the filter paper (Robinson, Price and Hogden, 1937). Albumin was taken to be the fraction remaining in solution after treatment with 21 per cent (w/v) Na_2SO_3. In this paper euglobulin and pseudoglobulin I, which together constitute the "immune globulin" of colostrum, are considered as one complex (Eu-I), viz. the portion precipitated by 18 per cent (w/v) Na_2 SO_3 at room temperature, and corresponding with the fraction of low electrophoretic mobility discussed by Smith (1948).

To test the response of the young calf to antigenic stimulus, some of the animals were given injections of a heat-killed *Salmonella dublin* vaccine.

RESULTS

General Clinical Observations

Table I presents a summary of the general clinical picture and also the weight of each calf. Calves which had received their dam's colostrum showed no scouring or other symptoms, and appeared normal in all respects. Calves deprived of colostrum could be divided into four groups according to their survival or otherwise and to the occurrence of scouring during the first week.

I (*died without scouring*). Both calves (10 and 13) in this group were found prostrate, became moribund and died within a matter of hours. Their faeces were sour, mushy and small in amount. A bacteraemia was revealed by blood culture in both animals.

II (*died after scouring*). In these animals (15, 27 and 30), scouring commenced at 1 to 2 days of age, displacing the meconium. Depression was acute, and no interest was displayed in food. Sinking of the eyes into their orbits was an early feature, although generalised subcutaneous dehydration was not pronounced except terminally. Weakness was progressive until death supervened.

III (*survived after scouring*). The initial symptoms were similar to those in the preceding group, although the onset of scouring was

sometimes delayed a little. The attack was less acute, and the scour either thickened gradually or ceased abruptly. Appetite and vitality were regained immediately on cessation of scouring. In some instances, particularly among the Shorthorn calves, diarrhoea recurred in the second and third weeks, but these attacks were milder.

IV (*survived without scouring*). In these calves the meconium was replaced by sour, mushy faeces, while appetite and spirits remained good. As in calves of the preceding group which had ceased to scour, progress was reasonably good unless fever or arthritis developed. The development of arthritis (joint-ill) in 7 of the 24 calves deprived of colostrum is the subject of a separate communication (Roberts, Worden and Rees Evans, 1954).

Pathological Observations

The following are brief notes on the calves that died or were killed:—

Calf **10**. Blood cultures at 46 and 66 hours yielded streptococci. Death occurred between the 79th and 89th hour. There was a patchy enteritis and an early broncho-pneumonia. Cultures from several sites gave a mixture of *Bacterium coli* and a streptococcus similar to that isolated by blood culture.

Calf **13**. A blood culture taken 4 hours *ante-mortem* showed a very heavy *B. coli* bacteraemia. The calf died at 58 hours. There was a dry peritonitis and an enteritis, chiefly of the ileum. *B. coli* was isolated in pure culture from several sites.

Calf **27**. Death occurred at 10 days. An enteritis, chiefly affecting the ileum, was found *post-mortem,* but there was no generalised bacterial infection.

Calf **30**. Death occurred at 11 days. A dry peritonitis and an ulcerative enteritis were present. *B. coli* was obtained from several sites.

Calf **15**. Blood cultures at 11 and 14 days were negative. Death occurred at 18 days. Autopsy showed a purulent omphalitis, multiple liver abscesses, and a patchy congestion of the intestine. The hock-joint contained excess cloudy fluid and fibrin clots. Culture from various sites showed a wide distribution of streptococci, together with diphtheroid organisms in the umbilical vein and abscess pus. The joint yielded a mixed growth.

Calf **22**. Killed at 13 days when still scouring. Patchy enteritis of the small intestine was found, but cultures from various sites were negative.

Calf **12**. Killed at 22 days when still scouring. An extra-abdominal purulent omphalitis, a patchy enteritis and bilateral focal interstitial nephritis were present. The joint lesions, previously evident clinically, had resolved. Cultures from various sites were negative.

Calf **6**. Killed at 49 days when apparently normal. The joint lesions noted earlier had resolved. A chronic purulent arteritis was present in one of the umbilical vessels and yielded a pure growth of *B. coli*. "H" and "O" antigens were prepared from this organism and were tested against the stored serum samples from this calf: the earliest sample with which

agglutination occurred was that which had been withdrawn at 29 days of age.

Calf **16**. Killed at 41 days when in a crippled condition. An advanced ulcerative arthritis of both stifles, with involvement of the internal iliac lymph nodes, was present, but cultures from these sites and from the joint fluids proved sterile.

Calf **31**. Killed at 59 days, by which time the left hind leg was useless. The stifle was affected by a marked ulcerative arthritis. Numerous leucocytes were present in smears of the joint fluid, but no bacteria were observed and none were obtained on culture.

Calf **19**. Killed at 91 days, by which time it had largely recovered from an earlier lameness. The only lesions found were fibrosis of the joint capsules and a slight excess of clear syovial fluid in the tibio-tarsal joint.

Calf **29**. This calf died during the night following vaccination on the 31st day. There were widespread petechial haemorrhages and the muscles were ischaemic. Cultures from various sites were negative.

The Faeces

In the newborn calf, fluid of amniotic type formed a pool in the stomachs and was distributed in small amounts throughout the small intestine. The caecum contained similar but thicker yellow-stained fluid. Typical meconium was yellow or green-brown and odourless, and consisted of firm rounded pellets that contained hair and were embedded in a tenacious mucoid matrix. At birth it was confined to the colon and rectum, becoming drier, with better-defined pellets, towards the anus. Stained smears of meconium did not reveal the presence of any bacteria.

When calves received colostrum, the resulting faeces gradually became mixed with the meconium during the first 12 to 24 hours and by about 48 hours had replaced it entirely. These faeces were yellow-orange, soft, sticky and tenacious, and had a rancid odour. Smears showed them to be rich in bacteria. They were replaced gradually by faeces derived from milk, which were yellow-brown, or sometimes khaki, sour and of a mushy consistency. In smears bacteria appeared to constitute the bulk of the latter faeces.

In calves deprived of colostrum and fed whole milk from birth one of three sequences was noted, viz., (i) the meconium was replaced directly by scour; or (ii) scour followed a mixture of meconium and sour faeces; or (iii) the milk-derived faeces replaced meconium directly. The scour was usually light yellow, the depth of colour depending on its water content. It often had a characteristic (not sour) odour, and smears showed the presence of numerous bacteria-containing phagocytes. The thicker type of scour often contained much mucus.

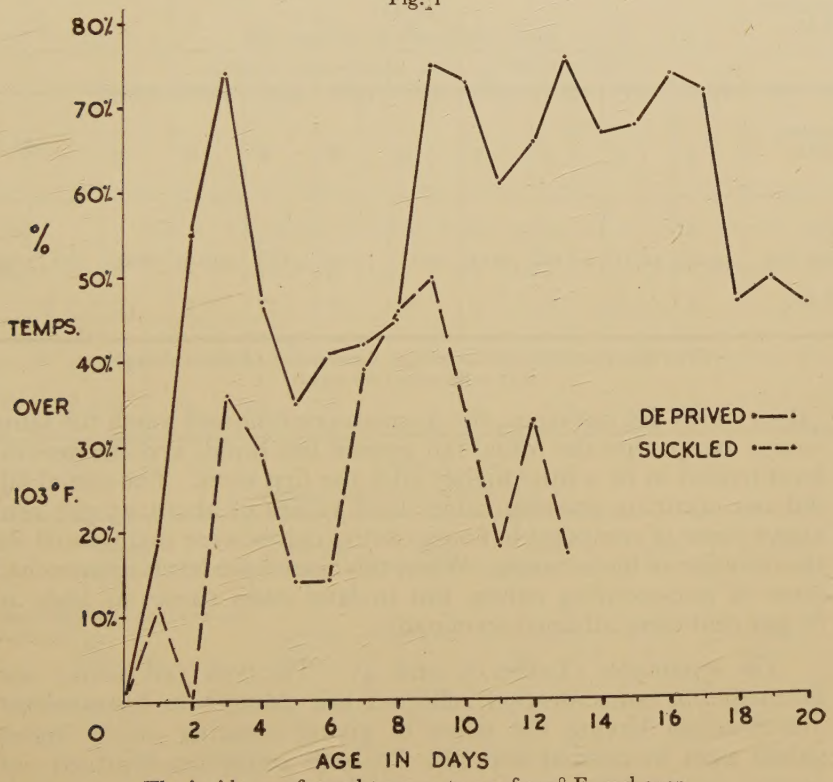
The presence of macroscopic amounts of blood in the faeces was a frequent finding in both control and deprived calves, whether scouring or not. It was especially common from 3 to 10 days of age, occurring as dark clots and also in a more diffuse lighter form.

During this period also mucus or mucoid strips, resembling casts of the intestinal mucosa, were often present.

Rectal Temperatures

The rectal temperatures showed considerable variation, not only between individual calves but also in the same animal. The general level in deprived calves was above that of the suckled, and in Fig. 1 the incidence of temperatures of 103°F. or over is recorded for comparison. Two peaks in temperature occurred in both groups those in the deprived group being the more pronounced. In these calves the first peak was correlated with the onset of scouring and was of short duration, whilst the second was often associated with malaise or joint-ill and was prolonged.

Fig. 1



The incidence of rectal temperatures of 103° F. and over.

Haematology

The packed cell volume (Table 2). Calves which received colostrum showed a fall in their P.C.V. of about 15 per cent on the first days, and then tended to show a gradual decline. This decline became more marked during the second week, when values fell to about 75 per cent of the level at birth. In the colostrum-deprived

TABLE 2

COMPARISON OF HAEMATOCRIT VALUES

Suckled and deprived calves, classified according to the occurrence of scouring during first week of life. (P.C.V. expressed as percentage)

AGE (days)	*0*	*>1*	*2*	*3*	*4*	*5*	*6*	*7*	*14*	*21*	*28*
Suckled		38(10)	38(8)	39(5)	39(8)	37(4)	37(8)	36(10)	33(9)	33(7)	34(3)
S.D.		5	7	3	4	5	4	5	6	5	1
Deprived Non-scourers	42(7)	40(4)	38(7)	37(4)	34(5)	38(5)	35(4)	36(7)	34(6)	35(5)	37(5)
S.D.	5	5	4	3	6	5	3	5	6	5	1
Deprived Scourers	45(17)	43(7)	47(11)	45(8)	49(6)	43(7)	45(5)	46(12)	40(13)		
S.D.	5	4	5	7	5	6	6	6	5		
All deprived	44(24)	42(11)	43(18)	42(12)	42(11)	41(12)	40(9)	42(19)	38(19)	37(14)	37(11)
S.D.	5	5	6	7	9	6	7	7	6	3	4

(The figures in parenthesis refer to the number of observations)
S.D.=Standard deviation

calves which did not scour, the haematocrit followed much the same course except that the initial fall seemed less rapid, and the general level tended to be a little higher after the first week. The initial fall did not occur in scouring calves, and values of about 25 per cent above those of comparable non-scouring calves were maintained for the duration of the scouring. When this ceased the levels approached those of non-scouring calves, but in fatal cases values as high as 60 per cent were attained terminally.

The erythrocytes (Tables 3 and 4). The red cell count and haemoglobin concentration reflected the changes in haematocrit, anhydraemia having the effect of giving scouring calves higher values than in normal animals. In both colostrum-deprived and control calves there was no appreciable change in mean corpuscular haemoglobin concentration (M.C.H.C.), but the cells tended to decrease slightly in size.

The leucocytes (Table 5). The total leucocyte counts in newborn calves showed greater variation than counts later in the first week, but they tended to be comparatively high. There was a slight but temporary rise following suckling, but in both colostrum-deprived

and control calves a fall in the total leucocyte count occurred during the first 3 to 4 days of life. Differential counts demonstrated that this decline was due to a fall in the number of polymorphonuclear cells, the numbers of mononuclear cells remaining fairly steady. While the neutrophils continued to diminish in numbers up to 6 or 7 days, there was a rise in the case of the mononuclears, the net effect on the total count being to keep it fairly constant, or even to raise it slightly in the case of the colostrum-deprived calves. Scouring did not markedly affect this first-week picture (Fig. 2), but elevated temperatures, with or without artiritis, which were common in the second week were always accompanied by a polymorphonuclear leucocytosis with counts of from 14,000 to 30,000/c.mm. Eosinophil cells were extremely few in numbers in all calves.

TABLE 3

ERYTHROCYTES (MILLIONS/C.MM.)

Suckled and deprived calves (as in Table 2)

AGE (days)	*0*	*>1*	*2*	*3*	*4*	*5*	*6*	*7*	*14*	*21*	*28*
Suckled		8·6(10)	8·5(7)	8·4(7)	8·8(5)	8·2(7)	9·0(3)	8·4(10)	7·9(9)	7·7(7)	9·3(3)
S.D.		1·0	1·1	0·9	0·7	0·7	0·9	1·0	1·1	0·9	0·6
Deprived	9·4(24)	8·9(11)	8·8(11)	9·0(11)	9·3(7)	9·0(8)	8·6(8)	9·1(20)	8·7(20)	8·6(14)	9·1(9)
S.D.	0·9	1·2	0·8	1·5	1·8	1·1	1·9	1·7	1·4	0·8	0·6

TABLE 4

HAEMOGLOBIN, M.C.H.C. & M.C.V.

AGE	*0–1 day*	*1 week*	*2 weeks*	*3 weeks*	*4 weeks*
Haemoglobin (gm./100 ml.					
Suckled Av.	10·7(10)	9·8(10)	9·1(9)	9·1(6)	10·0(4)
S.D.	1·2	1·2	0·9	1·2	0·8
Deprived Av.	12·2(24)	11·6(18)	11·0(18)	10·0(14)	10·3(11)
S.D.	1·3	1·6	1·4	1·4	1·2
M.C.H.C.(%)					
Suckled	28	27	28	28	29
Deprived	28	29	29	29	29
M.C.V. (cu. microgrms.)					
Suckled	44	43	42	43	37
Deprived	47	46	43	42	40

TABLE 5

TOTAL LEUCOCYTE COUNTS (THOUSANDS/C.MM.)

(as Table 2)

AGE (days)	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Suckled		12·8(10)	8·9(4)	8·6(5)	7·1(4)	7·8(4)	6·7(4)	7·2(6)
S.D.		3·3	1·2	2·2	1·9	1·4	1·1	1·6
Deprived Non-scourers	11·4(7)	8·9(4)	8·7(6)	7·2(4)	7·7(5)	6·7(4)	7·5(4)	7·6(6)
S.D.	4·6	2·0	1·7	1·6	1·1	1·6	0·9	1·2
Deprived Scourers	10·8(17)	11·5(5)	8·6(12)	7·3(6)	9·3(6)	9·1(6)	9·2(5)	8·5(9)
S.D.	2·2	2·6	2·3	2·7	2·7	2·1	2·8	2·7
All-deprived	11·0(24)	10·4(9)	8·6(18)	7·3(10)	8·6(11)	8·1(10)	8·4(9)	8·1(15)
S.D.	3·2	2·7	2·2	2·4	2·3	2·2	2·3	2·3

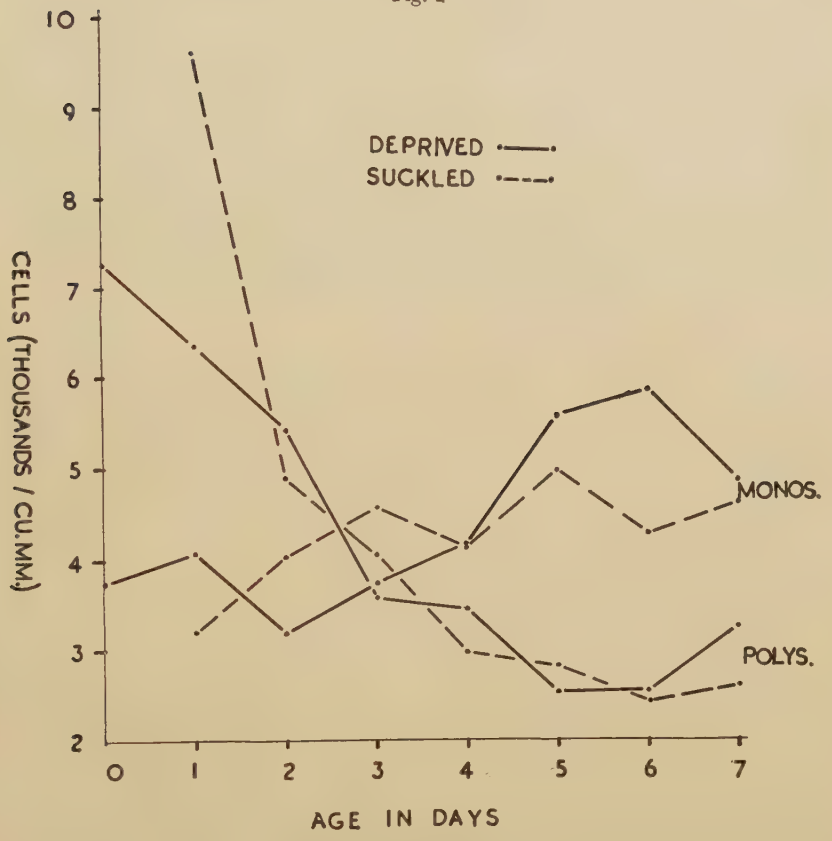
PERCENTAGE OF POLYMORPHONUCLEAR CELLS

Suckled		75	55	47	42	36	36	36
S.D.		10	8	9	6	4	4	6
Deprived Non-scourers	65	57	61	57	47	45	30	34
S.D.	11	11	13	8	9	10	15	15
Deprived Scourers	67	64	65	44	34	29	30	44
S.D.	7	16	12	11	12	9	5	11
All-deprived	66	61	63	49	40	31	30	40
S.D.	9	14	13	11	11	10	11	14

Plasma and Serum Proteins

Fibrinogen (Table 6). There was little change in the fibrinogen concentration in the plasma of control calves, while all deprived animals showed an increase, of the order of 100 per cent over the newborn value, by 4 to 5 days of age. Subsequent values depended on the degree of severity of the symptoms present. In those calves which had ceased to scour and which showed no pyrexia there was a decline as from 7 to 9 days of age, but in the others the rise continued until 13 to 15 days, the levels exceeding 1.00 g./100 ml. before declining. Values comparable with those of the control calves were attained at 15 to 30 days, except in those calves that developed arthritis, when a high concentration (up to 1.75 g./100 ml.) was maintained during the course of the disease (see Fig. 5).

Fig. 2



The inversion of the polymorphonuclear : mononuclear cell ratio.

TABLE 6

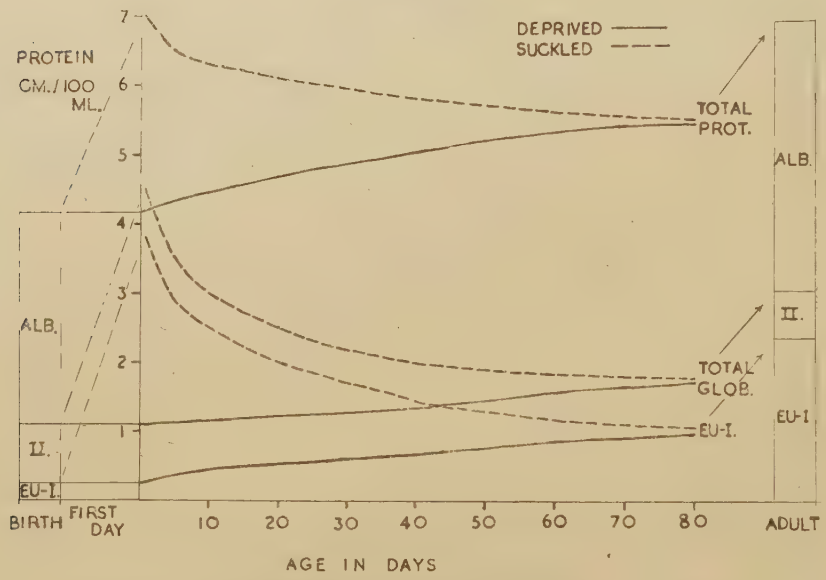
FIBRINOGEN (GMS./100 ML. PLASMA)

AGE days	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*14*	*21*
Suckled		0·40(10)	0·48(3)	0·43(5)	0·36(3)	0·40(4)	0·44(4)	0·40(5)	0·46(4)	0·38(2)
S.D.		0·06	0·07	0·09	0·06	0·12	0·11	0·06	0·07	0·03
De-prived	0·43(23)	0·46(12)	0·54(10)	0·61(11)	0·80(8)	0·83(10)	0·83(5)	0·91(6)		
S.D.	0·11	0·11	0·12	0·15	0·16	0·15	0·12	0·13		

Euglobulin-pseudoglobulin (Table 7). In the newborn unfed calf the serum characteristically contained little or no Eu-I fraction. Following the ingestion of colostrum, Eu-I appeared in the blood

serum, attaining a peak value at 18 to 24 hours and then declining. The rate of decline was at first considerable but then gradually slowed, and despite the wide individual variation in peak values a steady common level of 1.00-1.50 g./100 ml. was attained in all cases (Fig. 4). The initial peak concentration determined the age at which this level was attained in any particular calf. The calves deprived of colostrum and fed whole milk did not show any immediate rise in serum Eu-I, but if they survived and did not develop joint-ill the concentration of this fraction increased gradually, reaching about 1.00 g./100 ml. by 70 to 80 days of age (Fig. 3).

Fig. 3



The general serum protein picture.

Albumin (Table 7). The serum albumin in both control and colostrum-deprived groups had risen to about 20 per cent above the newborn value by 25 to 45 days of age. While this increase was however gradual from birth in the deprived animals, there was a preliminary fall in the case of the control calves. This preliminary fall in albumin concentration occurred parallel to the increase of colostrum globulin in the serum (Table 8), and the commencement of the decline in the level of acquired protein coincided with the rise in the concentration of albumin.

Pseudoglobulin II. This fraction changed little in concentration throughout the period of study, either in colostrum-deprived or control calves. There was possibly a very slight decline with age.

Total serum protein (Table 7). The total protein of the serum reflected the changes in the albumin and globulin fractions. There

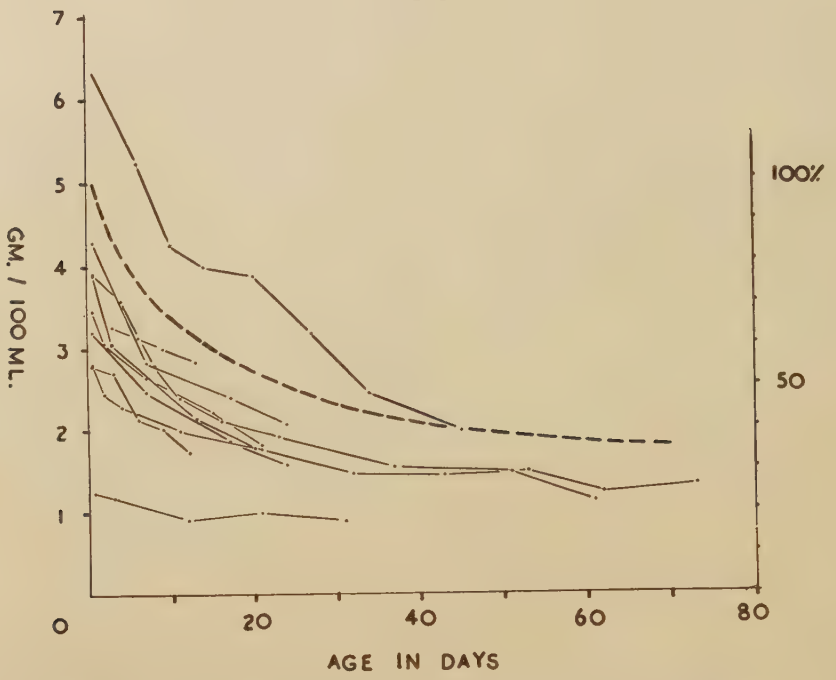
TABLE 7

SERUM PROTEINS OF DEPRIVED CALVES

(g./100 ml.)

AGE (days)	*0*	*2*	*9–11*	*14–16*	*19–21*	*24–26*	*28–32*	*35*	*40*	*45*	*50*	*55*	*60*	*70*	*80*	*Adult Cows*
No. of calves (and observations)	24	13	15	8	6	5	5	1	3	2	2	1	2	2	1	7
TOTAL PROTEIN SERUM	4·23 ± 0·30	4·12 ± 0·25	4·63 ± 0·24	4·70 ± 0·29	4·84 ± 0·20	4·84 ± 0·16	4·75 ± 0·10	5·00 ±	5·08 ± 0·12	4·93 ± 0·01	5·32 ± 0·17	5·40 ±	5·31 ± 0·10	5·35 ± 0·15	5·35 ±	6·95 ± 0·41
ALBUMIN	3·15 ± 0·28	3·10 ± 0·22	3·45 ± 0·19	3·51 ± 0·12	3·66 ± 0·13	3·62 ± 0·08	3·74 ± 0·04	3·70 ±	3·83 ± 0·12	3·64 ± 0·06	3·80 ± 0·18	3·75 ±	3·75 ± 0·13	3·77 ± 0·02	3·66 ±	3·90 ± 0·26
Eu-I	0·25 ± 0·16	0·28 ± 0·14	0·49 ± 0·17	0·51 ± 0·14	0·57 ± 0·14	0·64 ± 0·21	0·48 ± 0·19	0·73 ±	0·59 ± 0·09	0·66 ± 0·11	0·94 ± 0·08	0·84 ±	0·79 ± 0·09	0·99 ± 0·17	0·94 ±	2·37 ± 0·22
TOTAL GLOBULIN	1·08	1·02	1·18	1·19	1·18	1·22	1·01	1·30	1·25	1·29	1·52	1·65	1·56	1·58	1·69	3·05

Fig. 4



Euglobulin — pseudoglobulin I in suckled calves, the mean rate of decline (interrupted line) being expressed as percentage of peak values.

was a decline in the case of the control calves following an early peak, but the fall in Eu-I was offset to some degree by the rise in albumin. The fall in total protein was therefore not relatively as great as that in Eu-I, and a steady level of about 6.00 g./100 ml. was reached at 20 to 50 days of age. In the deprived calves there was a gradual rise from birth, due initially to both albumin and globulin and then mainly to globulin (Fig. 3).

TABLE 8

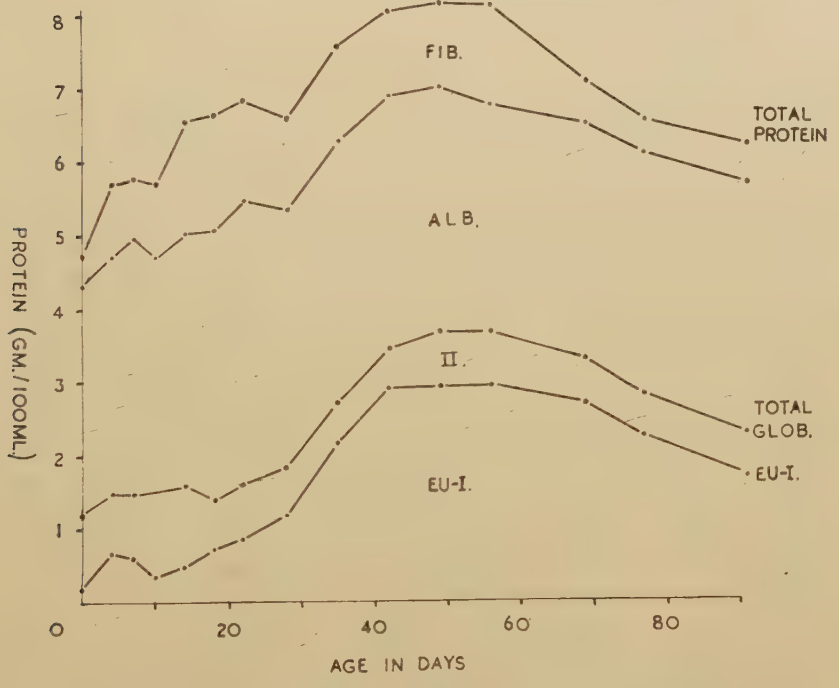
ALBUMIN CONCENTRATION

The Inverse Relation between Eu-I and Albumin concentration at or near the Peak Eu-I value in five Suckled Calves

Calf	*20*	*39*	*37*	*33*	*35*
Eu-I	6·35	3·44	3·20	2·81	1·22
Albumin	2·17	2·46	2·67	2·84	3·26

Serum proteins of "joint-ill" calves. Four calves (6, 16, 19 and 31) in which a chronic arthritis commenced at 9 to 15 days showed characteristic deviations from the pattern exhibited by the remaining colostrum-deprived animals. These differences took the form of an addition to the marked increase of fibrinogen noted previously. In each of the 4 calves the rate of increase of Eu-I became greatly accelerated at about 15 to 20 days, and by 30 to 40 days a value comparable with that of adult animals was attained and then exceeded. The total serum protein was also increased, but the albumin level was depressed slightly. In one calf (19, see Fig. 5), which was observed over a longer period than the others and which showed a fairly good clinical recovery, there was a decline in Eu-I from about the 60th day.

Fig. 5



The plasma protein picture in a calf (No. 19) with chronic arthritis.

Effects of Vaccination (Table 9)

Vaccination did not appear to influence survival nor to affect in any way the general picture seen in colostrum-deprived calves during the first week of life. In all cases each vaccination was followed by swelling at the site of inoculation, this reaction being present within 6 hours and persisting for 3 to 4 days. Depression and fever, accompanied usually by a leucocytosis, were evident at about 6 hours in

calves inoculated when 8 days of age or older. This reaction subsided in 3 to 4 days, and was generally less marked following subsequent inoculations. In calves that were inoculated when under 2 days of age there was little or no depression, fever or leucocytosis. A rise in fibrinogen concentration followed vaccination in all cases, and the first-week fibrinogen picture in vaccinated control calves resembled that in the unvaccinated colostrum-deprived animals. The appearance of antibodies did not appear to be related to previous consumption of colostrum, and in the case of both control and colostrum-deprived calves which were over 8 days of age when first inoculated flagellar ("H") antibodies appeared in from 3 to 13 days. Frequent vaccination of very young calves led to the production of "H" antibodies as early as the 6th or 7th day of life. In no case did the Eu-I level of the serum show any detectable change as a result of the appearance of the specific antibody.

DISCUSSION

The protective and other beneficial effects of colostrum-feeding have been amply demonstrated both in the field and experimentally. The protective action is put to greater test in experiments such as those described in this paper, in which colostrum derived from cows in different environments is found to exert its effect on calves kept under common experimental conditions (Briggs, 1951; Briggs, Lovell, Aschaffenburg, Bartlett, Kon, Roy, Thompson and Walker, 1951; Lovell, 1951).

The first week of life of the colostrum-deprived calf is characterised by a high incidence of scouring, and it appears to be the most critical, at least from the standpoint of survival. *B. coli* has been the organism most commonly incriminated in this early calf-mortality, largely on the evidence of *post-mortem* cultures from experimental calves. In field outbreaks, however, many calves presenting the typical clinical picture of scours during the first week do not yield any organisms on *post-mortem* culture of their organs. The explanation may lie partly in the fact that these animals, as distinct from experimental colostrum-deprived calves, receive sufficient protection from colostrum to prevent a generalised invasion but not sufficient to prevent a local, fatal infection. That other less luxuriantly growing organisms than *B. coli* may be involved at an earlier stage is indicated by the streptococcal bacteraemia seen in Calf 10, at 30 hours or longer before death. The role of bacteria other than *B. coli* is described also by Blakemore *et al.* (1954).

Scouring retards the normal gain in body weight through decrease of appetite, anhydraemia and impaired digestive function. The weight gains recorded in our colostrum-deprived calves during the second week of life, when scouring had ceased, may have been magnified as a result of restoration of the fluid balance. An increased resistance to the effects of deprivation was shown after the first week

TABLE 9

VACCINATION DATA

Leucocyte Response—
Total Leucocyte count and % Polymorphonuclears

Calf	*Breed*	*Col.*	*Age 1st Vacc.*	*Interval between vaccs.*	*No. of vaccs.*	*Age 1st 'H'*	*Age Max. 'H'*	*days from first vaccination*				
								– 1	*0*	*1*	*2*	*3*	*4*
28	F	D	42	10	4	48–1/10	67–1/320	—	59	69	78	—	63
4	X	D	37	10	2	40–1/40	44-1/2560		14·3	33·7	26·4		11·0
31	F	D	21	10	4	32–1/20	49–1/640	45	44	59	54	—	32
32	F	D	15	10	4	28–1/10	38–1/640	15·0	10·4	25·0	20·0		13·0
5	X	R	9	9	2	13–1/160	15-1/2560	—	31	73	52	—	41
36	F	D	8	8	2	14–1/160	20-1/2560	47	9·5	22·0	16·5		9·3
35	F	R	37 hrs.	10	3	18-1/1280	24-1/2560	7·8	38	57	—	—	47
34	F	D	29 hrs.	10	7	14–1/10	17–1/160	69	7·1	11·6			7·9
33	F	R	29 hrs.	10	7	15–1/160	17–1/320	11·2	62	54	—	38	
39	S	R	14 hrs.	3	3	7–1/40	13-1/2560	77	7·0	8·0		10·5	
40	S	D	2 hrs.	2	7	6–1/40	8-1/1280	15·5	58	67	—	—	39
								60	10·0	8·0			10·0
								10·6	76	66	—	44	—
								—	14·3	10·0		9·3	
									84	64	—	62	—
									19·0	7·0		6·0	

R — Received colostrum
D — Deprived colostrum

of life, and although arthritis was common the actual infection was probably temporary, the joint damage persisting as a sterile lesion in some cases (16 and 31).

The greater resistance of Friesian calves to the effects of colostrum deprivation noted by Blakemore *et al.* (1948), is supported by our evidence. Friesian calves also showed less severe symptoms than the Shorthorns, not only during the first week but also during the second and third weeks, when attacks of diarrhoea were common among the Shorthorns. The performance of our Shorthorn calves was nevertheless superior to that of the Jerseys and Ayrshires of Blakemore *et al.* (1954) and also to that of the Shorthorns of Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin and Lovell (1949). These differences in the results obtained with Shorthorns may have been due to environmental factors, including the provision in our case of a diet of whole bulk milk, or to the heterogeneous nature of the breed. Since our experiments were carried out Aschaffenburg, Bartlett, Kon, Roy, Sears, Ingram, Lovell and Wood (1952) have reported that the performance and survival of 12 Friesian bull calves deprived of colostrum did not differ significantly from those of 12 Shorthorn calves similarly treated, there being 11 deaths in each group. The calf house employed by these workers had, however, been used otherwise for a considerable number of experiments, and it appears likely that the degree of infection to which their calves were subjected was greater than in our own studies.

The first feeding appeared to contaminate the gut of the newborn calf. This is in accordance with the observations of Van Pelt, Johnson and Plastridge (1948), who found the meconium to be sterile and who demonstrated also the rapid establishment of bacteria in the intestine. Evidence of the importance of these bacteria in the aetiology of scouring is afforded by the fact that this can be prevented in the majority of colostrum-deprived calves by the prophylactic administration of antibiotics (Kastelic, Bentley and Phillips, 1950). The rapid growth of bacteria in the intestine probably shortly precedes the first peak noted in our temperature records and the crisis at 2 to 3 days observed by Kastelic, *et al.* (1950). In the absence of colostrum, which may exert an antibacterial effect in the lumen of the gut as well as following absorption, the intestine reacts and scouring ensues. The damage that is indicated by the presence of blood in the faeces may favour this process. Indeed the frequent occurrence of blood (McEwen, 1950) accompanied by mucoid spots in the faeces of colostrum-fed and deprived animals at from 3 to 10 days of age suggests that a shedding of the mucosa may occur. This is possibly a normal process associated with the unique permeability to protein of the calf's intestine during the early days of life (Comline, Roberts and Titchen, 1951 a,b).

The difference in temperature response to scours from that to arthritis may be due in part to the age incidence but is probably

associated with heat loss in the faeces. In fact the trough between the temperature peaks may be due largely to this factor.

The levels at birth of P.C.V., erythrocytes and haemoglobin are high by comparison with those recorded from adult cattle (Frazer, 1929; Ferguson, Irwin and Beach, 1945; Braun, 1946). The initial fall in all three values during the first day in calves that have received colostrum coincides with the appearance of the colostrum globulin in the serum, and is indicative of a small decrease in plasma volume. This fall is reduced or delayed when milk constitutes the first feed. Subsequently the presence or absence of scouring appears to be the main factor affecting the erythrocyte picture. The anhydraemia appears to have been relative rather than absolute, being best demonstrated by comparison of the values for scouring and non-scouring animals. The gradual decline in P.C.V. is seen to be due mainly to a diminution in the numbers of erythrocytes, the M.C.H.C. remaining constant and the M.C.V. falling only slightly. This downward trend over the first few weeks has been observed in normal calves by Frazer (1929) in the case of erythrocytes, and by Wise, Caldwell, Parrish and Phillips (1947) in the case of haemoglobin and P.C.V. The decline is temporary and probably corresponds with the "physiological anaemia of sucklings" discussed by Davidson and Leitch (1934), the deficiency being made good when solid feeding commences.

The significance of the neonatal leucocytosis is obscure, but it is a well-known phenomenon in the newborn baby (Garrey and Brown, 1935), and the fall in total count with inversion of the neutrophil-lymphocyte ratio has been observed also by Frazer (1929). Once the leucocytosis has subsided the total number and relative proportion of the white cell types correspond to those of adults. The absence of any marked difference in the leucocyte picture between the colostrum-deprived and control calves, despite the incidence of scouring in the former, suggests that during the first week of life the polymorphonuclear leucocytic responses are deficient. This is borne out by the inability of vaccination to elicit a leucocytic response during this period, whereas positive responses were obtained from later vaccinations.

The rise in fibrinogen concentration may be regarded as an attempt to increase the total plasma globulin in the globulin-deficient calves, but the similar rise in control calves that had been vaccinated soon after birth suggests that it may be a protective response to antigenic stimulation. The subsequent fibrinogen values provide a good indication of the occurrence of such reactions.

The general serum protein picture of the calves in these experiments is depicted graphically in Fig. 3. The very low level of Eu-I in the calf at birth may be compared with the adult level in which this fraction constitutes about one-third of the total serum protein. Following the ingestion of colostrum, the serum in the majority of

calves would attain Eu-I values above those of their dams, the actual level depending largely on the "quality" of the colostrum supplied. The decline in the concentration of the acquired globulin is a well-recognised phenomenon: our figure of a fall of the order of 50 per cent by 20 days of age agrees with that of Smith and Holm (1948) obtained electrophoretically, although the rate appeared to be diminished when the peak values were comparatively low (Fig. 4). During the first few days, loss via the urine may be a factor in this decline (Smith and Little, 1924; Howe, 1924). Later, however, dilution through growth is important, and must be balanced against the calf's own production of globulin. The common level to which the serum globulin of all the control calves declined is below that of adults, and it is probable that the adult value is not attained for a considerable period, even years (Garner, 1950). This suggests that normally the ability of the young calf to synthesise globulin is poor, and certainly in colostrum-deprived calves the increase in the level of serum globulin proceeds slowly (Howe, 1922; Hansen and Phillips, 1947; Smith, 1948). Our colostrum-deprived calves attained a Eu-I status comparable with that of the control calves at about 80 days of age, although other workers have demonstrated the persistence of colostrum globulin in the serum over a period of several months (Kerr and Robertson, 1943; McDiarmid, 1947; Smith and Holm, 1948). The fact that vaccination had little effect on the Eu-I levels of either colostrum-deprived or control calves, despite the appearance of agglutinins, emphasises the small proportion of the globulin that may be concerned with a specific immune property. This may be an important factor in assessing mortality and morbidity in experiments in which colostrum is withheld and milk is fed, since Smith (1946) has shown that about 10 per cent of ordinary milk whey protein consists of a globulin apparently identical with colostrum globulin. That this protein may be absorbed directly has been demonstrated by Hansen and Phillips (1949), who obtained an increased precipitin reaction against a purified colostrum pseudoglobulin antiserum with the serum of calves fed ordinary milk. The titres in response to vaccination obtained at 6 to 7 days indicate the age at which the calf can first produce its own antibodies, and this conforms with the observation that the highest calf mortality occurs during the first week of life.

Apart from its functions of conferring a passive immunity, the colostrum globulin, which contains all the essential amino-acids, may act as a valuable nutritional reserve for the rapidly growing calf. If the curves for the calves with the highest and lowest initial globulin levels are compared (Fig. 4), it will be seen that the advantages, immune and nutritional, would appear to favour the former over a considerable period. In this respect the possible effects of the practice of pre-milking, which reduces the globulin content of the, colostrum (Hill, Widdowson and Maggs, 1950; Aschaffenburg Bartlett, Kon, Roy, Walker, Briggs and Lovell, 1951) are evident.

The hyperglobinaemia seen in animals affected with chronic arthritis is comparable to that observed in man in certain chronic diseases (Gutman, 1948).

At birth the level of serum albumin is below that of adults. The initial fall in the serum albumin level in the control calves, coinciding with the appearance of colostrum globulin in the circulation and with the decline in the level of other blood constitutents, supports the view that there is a small change in plasma volume at this time. Albumin as well as globulin does however appear to be excreted by the kidneys for the first few days (Howe, 1924) and this may account for part at least of the decline in the albumin level. The adult level of serum albumin was attained at a relatively early age, there thus being a difference with the globulins, the low level of which seems to be mainly responsible for the lower total serum protein values encountered in young cattle (Garner, 1950).

Pseudoglobulin II constitutes at birth almost the whole of the serum globulin, and following a slight decline to the adult level this fraction remains remarkably constant, a fact that was noted by Howe (1922).

Since our experiments were carried out we have noted the further report from the Shinfield—Royal Veterinary College workers (National Institute for Research in Dairying, 1953), who have analysed the data relating to 156 calves kept in their experimental calf-house, and have shown that there is a frequency distribution characterised by two peak periods of scouring during the first 3 weeks of life, the first after the complete passage of the meconium and the other at 7 to 8 days of age. They have concluded further that the greater the quantity of colostrum fed during the first 24 hours of life, the lower the intensity of the post-meconial scour.

Blaxter and Wood (1953) have provided a valuable summary of the information relating to the incidence of scouring in calves that formed the subjects of their metabolic experiments. They have suggested that scouring is associated with high intestinal tract failure, and that bacteria play a secondary role by multiplying in the unabsorbed material present in the gut.

CONCLUSIONS

The effects of colostrum deprivation were observed in 12 Shorthorn, 8 Friesian and 4 other calves, the health and performance of which were compared with those of 12 control calves that were permitted to receive colostrum. There was a high incidence of disease (early scouring, later joint-ill) in the calves deprived of colostrum.

There was rapid establishment of bacteria in the gut following first feeding. Mucus and blood were commonly observed in the faeces between 3 to 10 days of age.

In calves deprived of colostrum there were two peaks in rectal temperature, occurring on or about the third and ninth days of life respectively.

The erythrocyte count of the blood fell gradually during the first 3 to 4 weeks of life in calves that had received colostrum, and in colostrum-deprived calves that did not occur. This fall was, however, delayed in scouring calves by the accompanying anhydraemia.

The inversion of the neutrophil-mononuclear cell ratio was not affected by colostrum deprivation.

The leucocytic responses of all calves during the first week of life appeared to be deficient.

Calves deprived of colostrum showed a marked increase in the blood fibrinogen level during the first few days of life.

In surviving calves deprived of colostrum there was a gradual rise in the euglobulin-pseudoglobulin I fraction of the serum. In calves receiving colostrum there was a continuous fall in the euglobulin-pseudogloublin I concentration from the early peak value, and by about 80 days of age the values recorded in colostrum-deprived and colostrum-fed calves were of approximately the same order. This level was, however, below that of adult cattle.

Calves suffering from joint-ill possessed high blood fibrinogen levels and developed a hyperglobinaemia.

Both colostrum-deprived and colostrum-fed calves appeared to be capable of producing their own antibodies as early as 6 to 7 days of age following repeated vaccination.

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ACTINOMYCOSIS AND TORULOSIS IN THE FERRET (*MUSTELA FURO* L)

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INTRODUCTION

Little has been written about diseases of the ferret (*Mustela furo* L.).

The common ailments of this animal have been described by Hughes (1947); tuberculosis (Symmers, Thomson and Iland, 1953) and neoplastic diseases (Symmers and Thomson, 1953) have been discussed in recent publications based on Dr. A P. D. Thomson's extensive experience with ferrets in the Department of Anatomy of the University of Birmingham Medical School ; and Momberg-Jørgensen (1952) has described a chronic disease of unknown aetiology, " enzootic malignant granulomatosis," which affected more than eleven per cent. of 1,227 ferrets examined in the Danish State Veterinary Serum Laboratory between January, 1948, and January, 1951, and which is still enzootic in the Laboratory three years later (personal communication to the authors).

We have found only one published record of any Mustelida with an infection for which a fungus may have been responsible : in this animal, a ferret, Levine, Dunlap and Graham (1938) found an intracellular parasite in the spleen, liver and lungs. Meleney (1940), when reviewing the few cases of human infection by *Histoplasma capsulatum* published up to 1940, drew attention to the likelihood that the ferret described by Levine, *et al.* (1938) was in fact infected with histoplasmosis. Indeed Levine *et al.* (1938) had themselves likened their parasite to those which Wenyon (1926) illustrated as *Cryptococcus farcinimosus* [*sic*] and as *Encephalitozoon cuniculi*. The organism to which Wenyon (1926) referred as a cryptococcus is in fact *Histoplasma farcinimosum,* the cause of epizootic equine lymphangitis.

Bennett (1944-45) drew attention to the fact that lymphangitis is not the only manifestation of this equine infection and he proposed that the disease should be called by the more general term *cryptococcus infection.* Unfortunately " cryptococcus infection " is itself an unsatisfactory term as current mycological practice requires that the name *Cryptococcus farciminosus* should be replaced by *Histoplasma farciminosum* (Medical Research Council Memorandum, 1949). *Equine histoplasmosis* might prove to be an acceptable substitute, the descriptive " equine " being essential in order to distinguish infection caused by *H. farciminosum* from infection by *H. capsulatum*, the organism implied when the unqualified name histoplasmosis is applied to disease in animals (Cole, Farrell, Chamberlain, Prior and Saslaw, 1953) and man (Conant, Smith, Baker, Callaway and Martin, 1954). Cryptococcus infection, or cryptococcosis, should be used only of

diseases caused by cryptococci, and even then the alternative name *torulosis* is at present preferred in the case of infection by the only important pathogenic cryptococcus, *C. neoformans* (Medical Research Council Memorandum, 1949).

Three cases of fungous infection in ferrets are recorded in this paper. Two are cases of actinomycosis and occurred in the colony of thirty-six ferrets which is kept for research purposes in the Canine Research Station of the Animal Health Trust. The third case is an example of torulosis.

REPORT OF CASES

Case 1.

Canine Research Station Animal-Index No. 1079. An apparently healthy female albino ferret, 18 months old, was bought from a dealer in January, 1953. It was caged with other ferrets until mated on the 20th February, after which it was isolated. Three healthy young were born on the 2nd April; they and the mate have remained well. Early in the course of its pregnancy 1079 was found to have developed two hard, tumour-like masses in the neck, one in the nuchal region and the other ventrally at the thoracic inlet. The overlying skin was thickened and adherent to the masses, and sinuses soon appeared over the dorsal lesion. The masses enlarged, and towards the end of the pregnancy the animal appeared to have some difficulty in breathing, although there seemed to be no dysphagia. Its general condition deteriorated after the litter was born and it died on the 14th April.

Post-mortem examination. The body was dissected immediately after death. Its weight (700 g.) was well within normal limits ; there was no emaciation. The teeth and jaws were healthy, and the pelt was normal except for the sinuses in the neck. The two cervical masses were completely separate from one another. The dorsal lesion, 5 cm. in diameter, was a dense, fibrotic mass, firmly adherent to the adjacent skin and muscles ; it was riddled with small abscesses containing thick, greenish-yellow pus which reached the skin surface through fibrous-walled sinuses. The second lesion, 3 cm. in diameter, was similar in appearance, but without sinuses. Neither lesion involved bone or viscera. Apart from some engorgement of the viscera there were no other abnormalities.

Bacteriological examination. Neither smears nor cultures were prepared from the cervical masses. Smears from the viscera contained no acid-fast organisms, and cultures from them on Löwenstein-Jensen medium were sterile.

Histological findings. Paraffin sections of formalin-fixed material were examined. The cervical masses consisted of organising granulation tissue, riddled with discrete or confluent abscesses. Neutrophile polymorphonuclear leucocytes predominated in the purulent exudate, although macrophages were also numerous. Each abscess was surrounded by young granulation tissue infiltrated by neutrophile polymorphs ; progressively more fibroblastic proliferation and fibrosis were apparent in the granulation tissue at greater distances from the centre of each suppurative focus, and there were many areas of dense sclerosis throughout the

lesions. Fibrosis extended into the surrounding tissues, but there was no effective encapsulation of the suppurative process which had spread into the nearby muscle and dermis, forming sinuses to the skin surface in the case of the larger lesion.

Colonies with the characteristic morphology of Actinomyces were easily recognised in the abscesses and sinuses in haematoxylin-eosin preparations (fig. 1) ; the largest colonies measured as much as 0.4 cm. in diameter. Gram's stain demonstrated the intertwined and branching threads of Gram-positive mycelium (fig. 2) ; characteristic club-like Gram-negative structures were seen at the surface of many of the colonies. No other organisms were demonstrated, either by Gram's stain or by the Ziehl-Neelsen stain ; the colonies contained no acid-fast elements.

Sections of the spleen showed an increased number of macrophages in the pulp, but the changes were not striking ; no organisms were found. There were no noteworthy changes in the liver.

Case 2.

Canine Research Station Animal-Index No. 1075. A male albino ferret, 12 months old, was bought in January, 1953, from the dealer who supplied 1079. It showed no sign of illness at any time, and its death on the 3rd April was unexpected. 1075 and 1079 had been caged together, and with other ferrets, before 1079 was mated.

Post-mortem examination. The only abnormalities which were found were in the lungs and mediastinal lymph nodes. There was considerable pneumonic consolidation in both lungs, with intervening areas of emphysema. About a dozen firm, white nodules were present beneath the visceral pleura in each lung ; the nodules were about 0.3 cm. in diameter, and appeared to be solid ; they did not project from the surface. The posterior mediastinal lymph nodes were enlarged to almost double the normal size ; their cut surface showed small abscess cavities filled with creamy pus in which minute greenish granules were visible.

Bacteriological examination was limited to a search for tubercle bacilli in scrapings from the pulmonary lesions and the mediastinal lymph nodes ; no acid-fast organisms were found in smears, and cultures on Löwenstein-Jensen medium were sterile.

Histological examination. Lung was the only tissue sectioned. The " nodules " proved to be acute abscesses containing small but morphologically and tinctorially typical colonies of Actinomyces. There was little formation of granulation tissue, and no fibrosis. No evidence of lipopneumonia was found.

The diagnosis in both these cases rests on the identification of the organisms by their morphology and staining reactions in histological preparations. The colonies, and the nature of the tissue-reaction, were so typical that the diagnosis of actinomycosis seems to be beyond doubt.

The source and portal of entry of the infection remain unknown, and speculation on these questions would be fruitless. No other cases of actinomycosis, and no diseases which in retrospect might have been actinomycosis, have been recognised in ferrets or in other animals, either in Kennett or in the stock of the dealer concerned.

It is worth recording that in life the tumour-like masses of granulomatous tissue in ferret 1079 suggested a diagnosis of neoplasia. Two other conditions which we have seen in ferrets might have been considered in the differential diagnosis : one of us (W.St.C.S.) has seen a number of ferrets with granulomatous masses and sinuses in the cervical region due to myiasis, the larva concerned being that of the common warble-fly, *Hypoderma bovis;* a similar picture is presented by staphylococcal or streptococcal cervical cellulitis complicating mandibular osteomyelitis associated with dental sepsis, a condition akin to Ludwig's angina in man.

The subpleural actinomycotic nodules in the lungs of ferret 1075 macroscopically resembled the focal lipopneumonia of uncertain aetiology which is often seen in apparently healthy ferrets at necropsy (Symmers, Thomson and Iland, 1953).

Case 3.

A mature male albino ferret, privately owned, was unexpectedly found dead in its hutch.

Post-mortem examination. Only the alcohol-fixed abdominal viscera were available for examination. The pelt and thoracic viscera were said to have been healthy. The serosal surfaces of the abdominal organs were thickly covered with colourless, jelly-like material which was easily peeled off the underlying serosa ; there were extensive fibrous adhesions between the viscera. A few firm, white nodules, about 0.1 cm. in diameter, were present on the serosal surface of the intestines. The mesenteric lymph nodes were moderately enlarged ; their cut surfaces showed scattered nodules similar to the subserosal lesions. Identical nodules were sparsely scattered in the spleen and liver, which were not enlarged.

Histological examination. The jelly-like material coating the peritoneal serosa consisted of masses of yeast-like organisms, each about 10 μ in diameter and surrounded by a mucoid capsule of about the same width (fig. 3). The capsule had a strong affinity for mucicarmine, stained metachromatically with toluidine blue and gave a positive periodic-acid-Schiff reaction. A few leucocytes, mostly neutrophile polymorphs, were present amongst the yeast cells. Identical organisms were present in small numbers in the cytoplasm of macrophages and multinucleated giant cells which formed small tubercle-like granulomata, corresponding to the nodules seen macroscopically in the serosal surface of the intestines, in the lymph nodes and on the spleen and liver. The morphology and staining-characteristics of these yeast-like organisms were typical of cryptococci.

The appearance of the organisms in Case 3, and the nature of the tissue reaction, which was of the type seen in natural and experimental cryptococcal infection in other species, justify the diagnosis of torulosis (true cryptococcosis). Torulosis is a well-known, if uncommon, infection in man (Cox and Tolhurst, 1946) ; it has seldom been reported as a spontaneously occurring disease in animals, and in this respect it should be borne in mind that cryptococcosis is a misnomer when used as a synonym for equine histoplasmosis (epizootic lymphangitis). Torulosis has so far been

recognised in the ox (Sanfelice, 1895), the pig (Sanfelice, 1898), the horse (Frothingham, 1902 ; McGrath, 1954 ; also, the cases of Meyer and of Weidman and other possible cases referred to by Saunders, 1948), the cheetah (*Cynaelurus jubatus*) (Weidman and Ratcliffe, 1934 ; Ratcliffe, quoted by Saunders, 1948), the domestic cat (Holzworth, 1952 ; Holzworth and Coffin, 1953 ; McGrath, 1954), the dog (Seibold, Roberts and Jordan, 1953 ; McGrath, 1954) and the marmoset (*Leontocebus geoffroyi*) (Takos and Elton, 1953) Torulosis has also been recognised in the guinea-pig (Dezest, 1953). Pathogenic cryptococci have been isolated from cow's milk (Klein, 1901 ; Carter and Young, 1950), apparently in the absence of detected disease in the herds providing the supply ; and *C. neoformans* has recently been incriminated as the cause of outbreaks of bovine mastitis (Pounden, Amberson and Jaeger, 1952 ; Innes, Seibold and Arentzen, 1952 ; Simon, Nichols and Morse, 1953).

CONCLUSIONS

Two cases of actinomycosis in the ferret (*Mustela furo* L.) are described. A case of torulosis in the ferret is also reported. These infections have not been reported before in any Mustelida.

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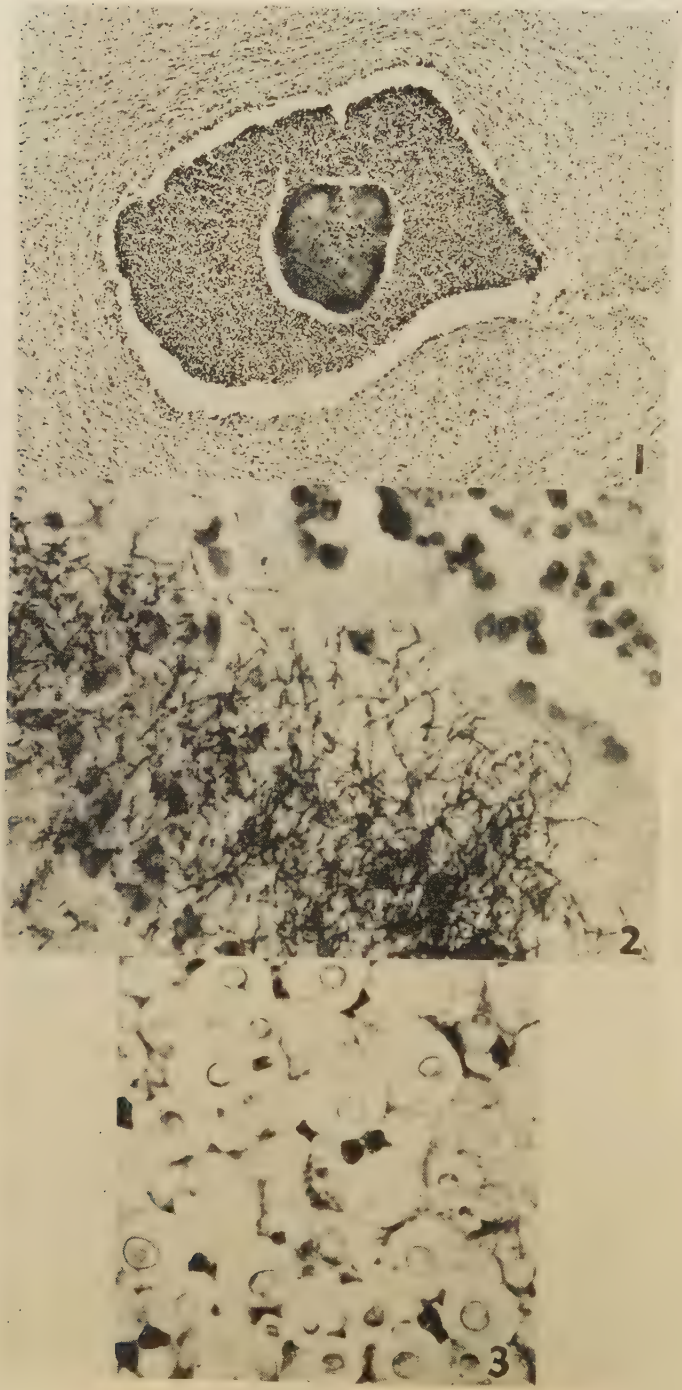

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LEGENDS TO FIGURE

Fig. 1. *Case 1.* Small abscess cavity in one of the cervical granulomata, with a colony of Actinomyces in the pus. (Mayer's haemalum and eosin; X 70.)

Fig. 2. *Case 1.* Margin of a colony of Actinomyces, showing mycelium. Pus cells in upper right-hand corner. (Gram's stain; X 690.)

Fig. 3. *Case 3.* Part of the jelly-like coating on the peritoneum, showing typical cryptococci. The wide, clear space around each organism is its mucoid capsule. A few leucocytes are present. (Mayer's haemalum and eosin; X 430.)

OBSERVATIONS ON THE SYSTEMIC TUBERCULIN REACTION IN THE TUBERCULOUS GUINEA PIG.*

By

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INTRODUCTION

The mechanism by which tuberculin produces its deleterious effect in the tuberculous animal is still only imperfectly understood, but it is generally accepted that the systemic tuberculin reaction is a manifestation of bacterial hypersensitivity and depends, at least in some degree, upon the union of an antigen in tuberculin with a cellular antibody (Rich, 1951). The first recorded account of the systemic tuberculin reaction in the guinea pig is attributable to Koch (1891), who also described the characteristic autopsy findings in animals that had died of tuberculin shock. The present paper embodies a study of the effects of parenterally administered tuberculin on the tuberculous guinea pig, and it was carried out in an attempt to gain a closer insight into the nature of bacterial hypersensitivity.

MATERIALS AND METHODS

The Experimental animals. The guinea pigs in these experiments were obtained from several different breeders. They were albino or Himalayan, of either sex, usually weighing 250 to 400 grammes. They were maintained on a diet of damp mash composed of bran 50 per cent oats, 25 per cent, grass meal 15 per cent and meat and bone meal 10 per cent supplemented by a good ration of hay and green food.

Mode of infection with tuberculosis. With very occasional exceptions, tubercle bacilli of bovine type were employed throughout. Three strains were available :— Strains 3076 and VPL were of approximately similar virulence and intramuscular doses into the thigh of 0.001 mg. (semi-dry weight) produced death after 12 to 14 weeks. Strain IAP was rather more virulent and a similar dose killed the animals after about 10 weeks. Strain 3076 was grown on Herrold's egg medium and the other strains on Lowensteins medium. Suspensions in saline containing 0.001 to 0.020 mg. were injected intramuscularly into the inner aspect of the right thigh or occasionally intraperitoneally.

The tuberculin. Purified Protein Derivative Tuberculin (PPD), as issued by the Ministry of Agriculture Veterinary Laboratory at Weybridge for the routine testing of cattle, was employed throughout. Weybridge PPD tuberculin is a trichloracetic acid precipitated fraction prepared from heated cultures of human type tubercle bacilli (Green, 1946). The dry powder contains approximately 5 per cent of polysaccharide and is issued as a 2 mg./ml. solution in M/15 phosphate buffer, with 10 per

* The observations contained in this paper formed part of a thesis submitted to the University of Liverpool in fulfillment of the degree of Ph. D.

cent glycerol and 0.5 per cent phenol. Each millilitre therefore contains approximately 0.1 mg. of polysaccharide.

The intradermal tuberculin test. 320 i.u. of PPD dissolved in 0.2 ml. of sterile saline was injected intradermally into the depilated flank, using a 26-gauge, or a very fine dental needle. The reactions were read after 24 hours when the extent of erythema, necrosis (pallor) and central haemorrhage were measured. The degree of induration was assessed by palpation.

To produce the systemic tuberculin reaction, 2 mg. of PPD (1 ml.) was injected intramuscularly into the inner aspect of the left thigh or occasionally intraperitoneally. The effect of the systemic reaction on the clinical condition, rectal and skin temperatures, blood pressure and haematological picture was studied.

Temperature recording. Rectal temperatures were recorded with an ordinary clinical thermometer, gently inserted 3 to 5 cms. into the rectum and retained until the reading was steady. Skin temperatures were obtained with a calibrated thermocouple connected to a sensitive galvanometer. For this purpose, the guinea pigs were placed on a special stand, described and illustrated by Miles and Niven (1950). The thermocouple was attached to the floor of the platform of the stand, so that when the animal was in position, its belly pressed firmly against it. The animals were retained on the stand for several minutes until the galvanometer reading was quite steady.

This method gave reliable and reproducible results.

Blood pressure recording. The blood pressure during the systemic tuberculin reaction was recorded in the small arteries of the ear, using the modified Grant's Capsule devised by Miles and Niven (1950). After applying the capsule to the ear, vasodilatation of the selected artery was induced by repeated raising and lowering of the intracapsular pressure ; it was facilitated by previous warming of the ear. The shocked animals required a longer application of these measures than the unshocked. When satisfactory vasodilatation had been achieved, the pressure necessary just to prevent flow along the artery was determined in 5 to 20 successive readings, whose mean was then computed.

Haematological procedures. Blood samples for haematological examination were obtained by puncture of the small veins in the transilluminated ear, after discarding the first drop of blood. There was usually little struggling. Before taking blood for platelet counts, the ear was depilated with barium sulphide paste. The total leucocyte counts were performed on at least duplicate samples from a carefully filled pipette, using, as a rule, Neubauer counting chambers. From each blood sample, two films were prepared and stained by Leishmann's method. The differential counts were made on at least 200 leucocytes. From the total and the differential counts, the actual numbers of (i) neutrophils, (ii) lymphocytes + monocytes, per cubic millimetre, were computed for each sample. For purposes of comparison, these absolute counts were each standardised so that the respective pre-injection values represented 100. Subsequent variations above or below were then shown as percentages of this. The respective results for the three counts (total, neutrophil and non-granular cell) in each group of guinea pigs are presented as mean percentages of the pre-injection values.

The platelets were counted directly, using blood diluted 1 : 100 in

filtered 3.8 per cent sodium citrate solution in a 'red cell counting' pipette. All counts were performed in duplicate.

Method of destruction and time of autopsy. The guinea pigs were killed by a blow on the head, or occasionally by ether or coal gas. Autopsies were carried out at once or, in those dying overnight from tuberculin shock, the following morning. The guinea pigs with febrile reactions were destroyed 24 hours after injection of the PPD.

Histological techniques. Material for histological examination was obtained immediately after death whenever possible and fixed in Bouin's Solution, except for the adrenals and kidneys for which 10 per cent formalin was used ; occasionally formalin was used as the fixative for all the tissues. After dehydration in alcohol, etc. and embedding in paraffin wax, sections were cut at a thickness of 6 microns and stained routinely with haematoxylin and eosin, and, whenever indicated, by the Giemsa Ziehl-Nielsen, or Picro-Mallory methods.

Experiments on the role of histamine. Samples of blood for estimation of histamine were prepared and assayed according to the method of Code (1937), using the atropinised guinea pig ileum. The blood extracts were inactive if the gut had previously been treated with a dose of mepyramine maleate sufficient to render it insensitive to a standard dose (0.2μg.) of histamine acid phosphate. In the lung perfusion experiments, the technique of Feldberg and Kellaway (1937) was adopted. The PPD used for perfusion was either dissolved in the perfusion fluid (Tyrode's Solution), or alternatively, was injected through the rubber tubing at the inflow canula into the interior of the pulmonary vasculature ; backflow was prevented by a proximal clip and perfusion was discontinued for 5 minutes. The histamine content of the venous perfusate was assayed after $\frac{1}{4}$, 1, 2, 3, 4, 5, 6, 7 and 8 hours according to the duration of the experiment. The perfusate was boiled briefly and allowed to cool before the assay was performed.

RESULTS

The Clinical Effects of PPD in Normal and Tuberculous Guinea Pigs

The Effect of PPD in Normal Guinea Pigs

The rectal temperatures of 10 normal tuberculin-negative guinea pigs were recorded hourly during an 8 hour experimental period anp again at the end of 24 hours. The results in each animal were expressed in terms of the departure from the initial value (0 hours), and the average temperature fluctuations for the group were then calculated for each hourly observation period (Table I). There were wide variations in the rectal temperatures of different animals, and sometimes even in serial observations on the samc animal.

After an initial temperature recording, the same guinea pigs were then injected intramuscularly with 2 mg. of PPD and the rectal temperatures were again observed as before. The mean temperature variations at each observation period were then compared with the previous values obtained when the guinea pigs were uninjected. In no instance was there any statistical difference. The tuberculin produced no detectable clinical effects in these guinea pigs, which remained quite normal throughout the experiment.

TABLE I

THE TEMPERATURE CHANGES (°F) IN NORMAL AND TUBERCULOUS GUINEA PIGS

Group Hrs. →	0	1	2	3	4	5	6	7	8	24
Untreated normal guinea pigs	0	+0.37 ± 0.54 10	+0.49 ± 0.54 10	+0.58 ± 1.00 10	+0.60 ± 1.09 10	+0.60 ± 1.10 10	+0.59 ± 1.32 10	+0.54 ± 1.15 10	+0.55 ± 0.96 10	+.078 ± 1.18 10
Normal animals treated with 2 mg PPD.	0	+0.18 ± 0.77 10	+0.44 ± 0.56 10	+1.46 ± 1.13 10	+1.34 ± 1.24 10	+0.85 ± 1.27 10	+0.57 ± 1.08 10	+0.21 ± 1.05 10	+0.35 ± 1.11 10	+0.25 ± 1.04 10
Untreated tuberculous guinea pigs	0	+0.02 ± 0.53 5	+0.06 ± 0.29 5	−0.08 ± 0.35 5	+0.04 ± 0.34 5	−0.16 ± 0.47 5	−0.24 ± 0.46 5	−0.18 ± 0.38 5	−0.26 ± 0.22 5	+0.10 ± 0.39 5
Guinea pigs with tuberculin fever.	0	+1.14 ± 0.92 13	+2.71 ± 1.29 14	+3.79 ± 0.83 14	+3.67 ± 1.35 12	+3.35 ± 1.26 14	+2.95 ± 1.18 13	+2.68 ± 1.22 12	+3.64 ± 1.22 9	+0.66 ± 1.22 13
Guinea pigs with non-fatal shock	0	+0.95 ± 0.93 4	+1.76 ± 1.18 5	+1.97 ± 0.80 4	+1.63 ± 1.15 3	+0.37 ± 0.87 4	−0.54 ± 1.18 5	−1.57 ± 0.67 4	−1.52 ± 0.69 4	−1.46 ± 2.24 5
Guinea pigs with typical tuberculin shock	0	+0.71 ± 1.51 13	+1.49 ± 1.11 13	+0.87 ± 1.80 12	−2.08 ± 2.45 13	−4.05 ± 2.45 11	−6.04 ± 2.18 10	−8.17 ± 2.46 7		

The figure below each value indicates the group size.

The Significance of Differences between the Groups : Values of P (Level of Significance. P—0.05)

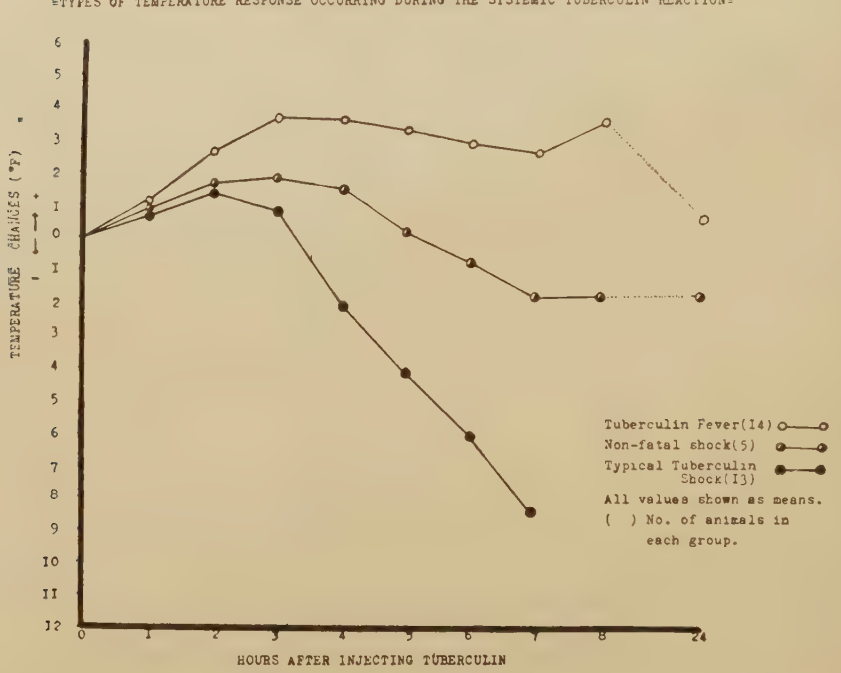
Group Hours——>	1	2	3	4	5	6	7	8	24
Normal animals untreated Same animals given PPD	>0.1	>0.1	>0.05	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
Normal, given PPD. Animals with tuberculin fever.	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.1
Normal, given PPD. Non-Fatal Shock	>0.1	<0.05	>0.1	>0.1	>0.1	>0.05	<0.001	<0.05	>0.05
Normal, given PPD Tuberculin Shock	>0.1	<0.05	>0.1	<0.001	<0.001	<0.001	<0.001		

The Effect of Tuberculin in the Tuberculous Guinea Pig

The constitutional disturbance following the injection of PPD into tuberculous guinea pigs may assume several different forms according to the prevailing experimental conditions.

The simple febrile reaction. This form of reaction consisted of a sharp rise in body temperature, usually commencing within 1 hour of injection and rising after 3 to 4 hours to a peak of 3 to 5°F above the initial value. This was followed by some decline but sometimes there was a minor secondary rise at 8 hours. By 24 hours after injection, the temperature had fallen to its initial value or a little above. Temperature responses of this type are shown in Figs. 1

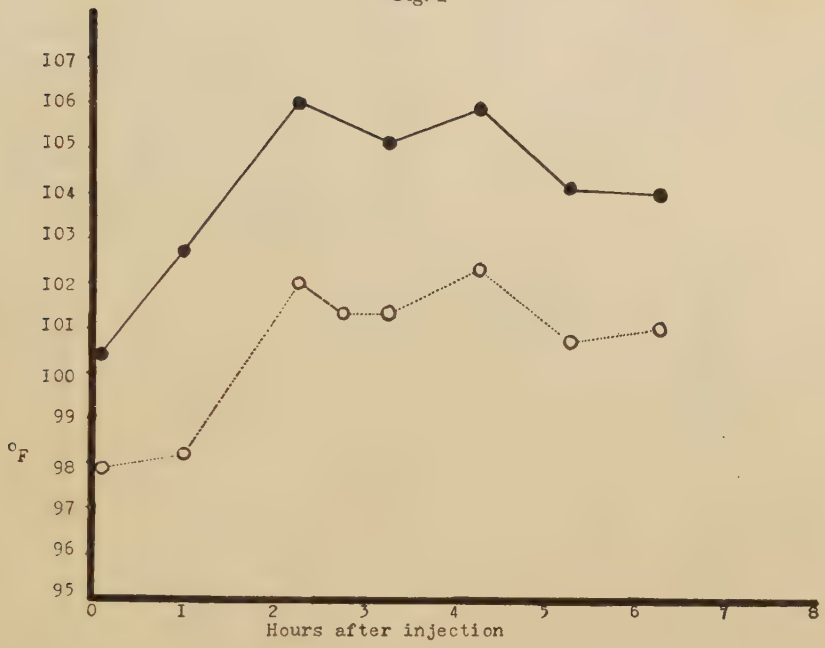
Fig. 1


TYPES OF TEMPERATURE RESPONSE OCCURRING DURING THE SYSTEMIC TUBERCULIN REACTION
TEMPERATURE CHANGES (°F)
HOURS AFTER INJECTING TUBERCULIN
Tuberculin Fever(14)
Non-fatal shock(5)
Typical Tuberculin Shock(13)
All values shown as means.
( ) No. of animals in each group.

and 5. The temperature values observed during this reaction were significantly higher than in tuberculin-injected normal animals (Table 1). The skin temperature ran closely parallel with the rectal temperature (Fig. 2). The animals often shivered and were slightly less active than usual, but they still struggled vigorously when handled and the appetite remained unimpaired even at the height of the reaction. There was no marked alteration in blood pressure (Fig. 3) and by 24 hours after injection, the animals were quite normal again.

Tuberculin shock. In the guinea pigs developing tuberculin shock, there was usually a preliminary temperature rise commencing within

Fig. 2

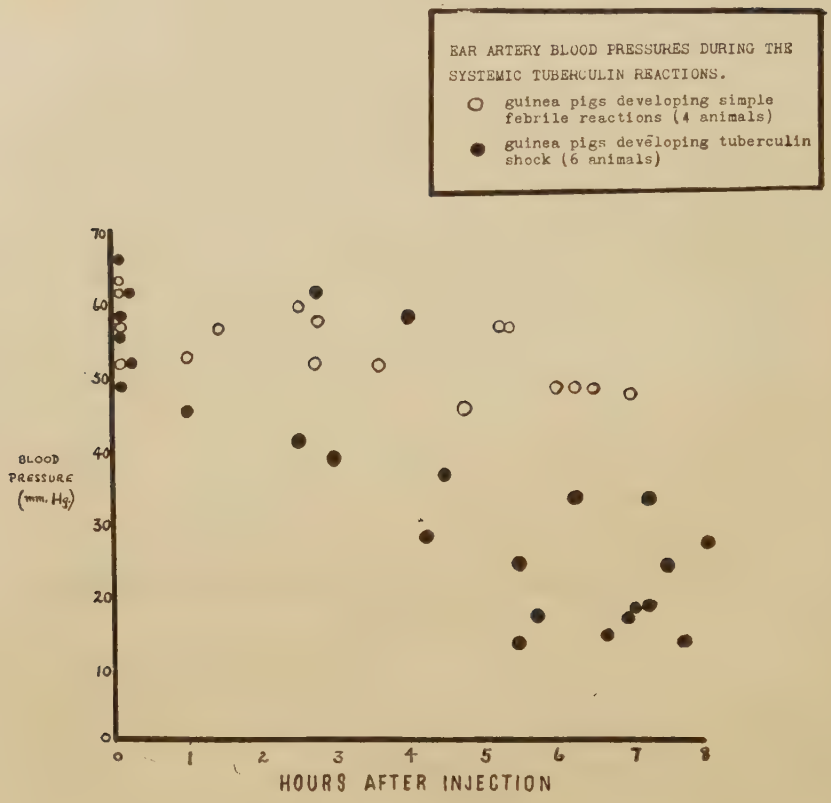


The Relation between Skin and Rectal Temperatures in a Guinea Pig which developed a Simple Febrile Reaction following the Injection of PPD.

●——● Rectal Temperatures
o----o Skin Temperatures.

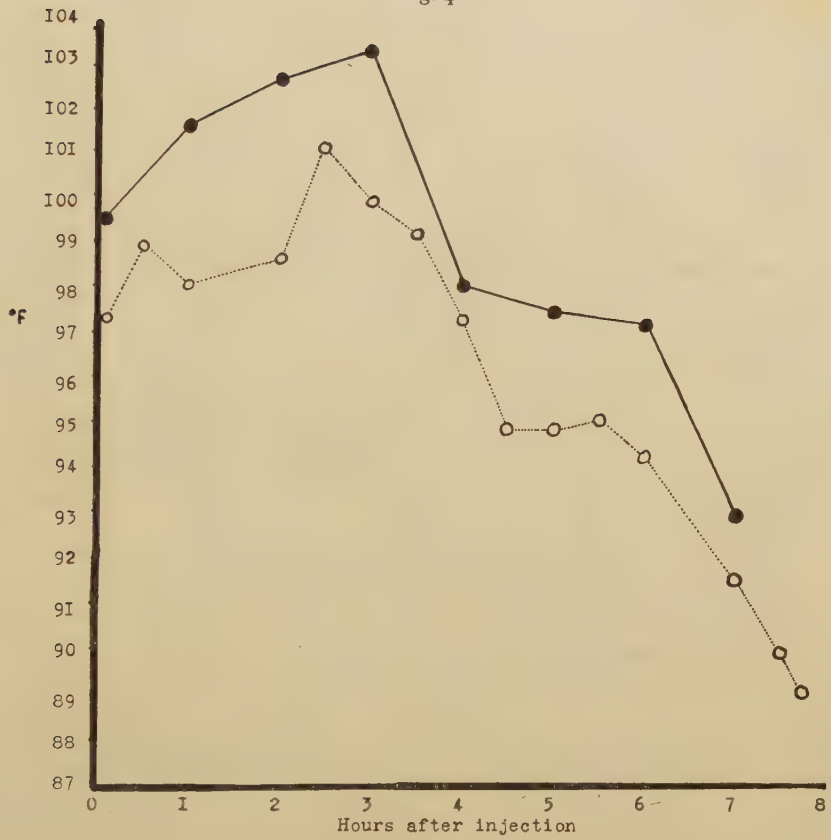
1 hour of injection and reaching a maximum after 2 hours. The latter was significantly less than in the guinea pigs showing simple febrile reactions, but higher than in the PPD-injected normal controls. During this preliminary febrile stage, there was no apparent clinical difference between the guinea pigs in this group and those in the last, although a few animals appeared unduly dull even at this early stage. About 2 to 3 hours after injecting the PPD, the temperature commenced to fall and the first symptoms of shock appeared. From then onwards, the temperature fell progressively until death, by which time readings were unobtainable with the ordinary type of clinical thermometer. In the mean temperature curve of 13 tuberculin shocked guinea pigs, the average decline in rectal temperature was practically linear with time (Fig. 1). The fall in skin temperatures reflected fairly closely the decline in rectal values, although the parallelism was less close than in the animals in the last group (Fig. 4). The symptoms of shock accompanying the decline in body temperature were likewise progressive. In their earliest stages, they were confined to dullness, inappetance, disinclination to

Fig. 3



move, and elevation of the hair, particularly around the nape. With the deepening of shock however, other symptoms appeared, of which the most important was a pronounced muscular weakness. The head seemed to sag, and when the animal was induced to move, the weakness of the hind legs was revealed by a tendency to drag the belly along the floor during progression. On handling the animal was chilly and flaccid, and when replaced in the cage would often rock to and fro as if off balance. The guinea pig would become progressively slower in righting itself when placed in an abnormal posture and only after 10 to 20 seconds would it make any attempt to regain its normal position. If left undisturbed, it assumed a huddled attitude, with eyes half closed, and appeared for the most part to be unresponsive to its environment. Respirations were usually increased and there was a marked fall in blood pressure. Examination of the vessels in the transilluminated ear with a lens revealed vasoconstriction, and segmental arterial contractions of the type described by Delaunay, Boquet, Lebrun, Lehoult and Delaunay (1948) in endotoxin-shocked rabbits. Eventually weakness would become so

Fig. 4



The Relation between Skin and Rectal Temperatures in a Guinea Pig which developed Tuberculin Shock following the Injection of 2mg. of PPD.

●——● Rectal Temperatures

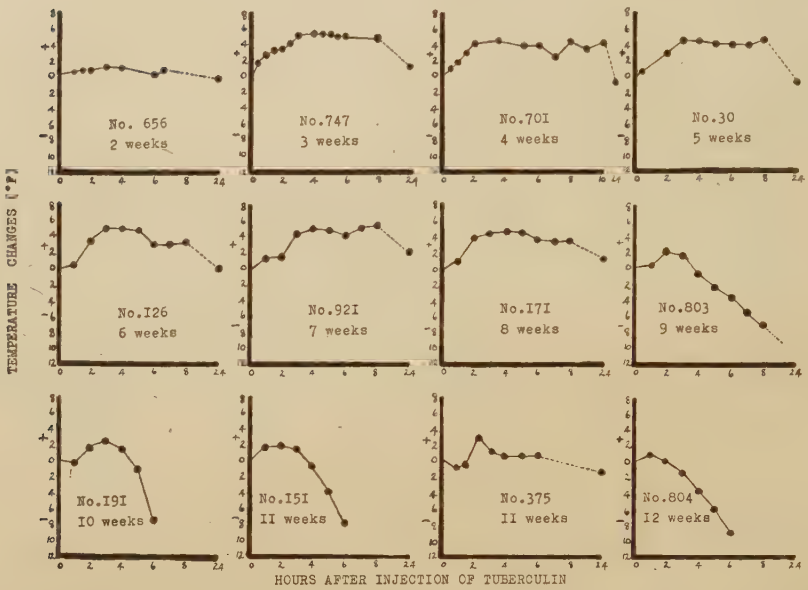
○------○ Skin Temperatures

marked that there would be complete loss of postural tone and the animal would fall on its side. The final stage of the picture consisted of intermittent convulsions, disappearance of reflexes and gradual loss of consciousness. The convulsions took the form of violent spasmodic twitchings of the whole body, running movements of the forelegs and spasmodic kicks of the hind, sometimes accompanied by opisthotonus. There was loss of the corneal reflex, and in one animal, pupillar inequality, loss of pupillo-motor reflexes and downward rotation of one eyeball. Respiration deteriorated into intermittent gasps and finally the heart would cease to beat. Death occurred 4 to 30 hours after injecting the tuberculin.

The development of tuberculin shock was accompanied by

Fig. 5

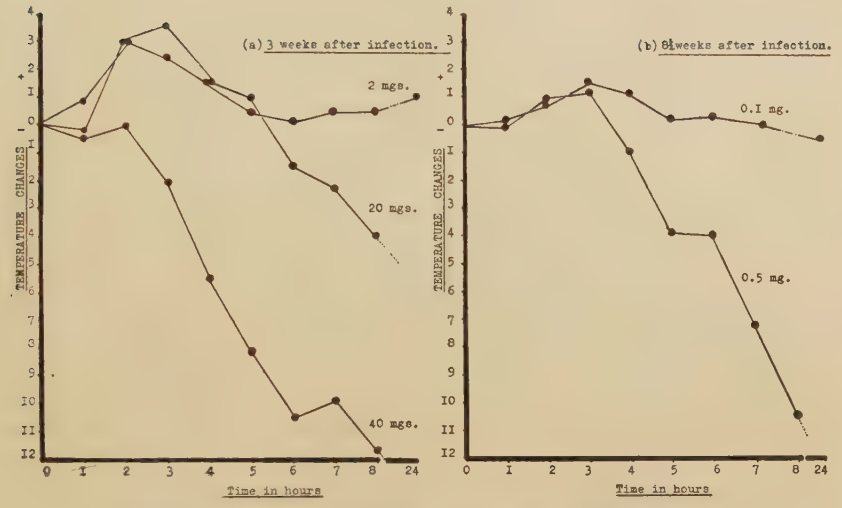
Temperature curves produced by PPD in tuberculous guinea pigs(350-400 gms.) which had been infected 2-12 weeks with 0.001mg. bovine tubercle bacilli.



striking alterations in the appearance of recent skin test sites. These are of some interest as reflections of the behavior of the cutaneous vessels during shock. In guinea pigs skin tested during the previous 24 to 48 hours, it was found that once the rectal temperature commenced to fall, the erythema at the skin reaction site began to fade and rapidly disappeared completely, leaving the previously erythemic skin indistinguishable in colour from the surrounding normal tissue. With the disappearance of the contrasting erythema, the pallor associated with areas of necrosis became difficult to see. Sometimes slight fading of the erythema seemed to be evident even before shock had actually appeared, but this was usually somewhat equivocal. It seems reasonable to attribute this fading of the inflammatory erythema to peripheral vasoconstriction in response to the development of shock. When the reaction site was studied for a further 2 to 3 hours, a faint purplish tinge became apparent at the sites from which the erythema had disappeared, and this extended and intensified until by the time of death, the whole of the area had become a deep purple. This discolouration was limited to the 'erythemic zone' of the skin reaction and was apparently due to intense engorgement of the local vessels in the late stages of shock.

Non-fatal tuberculin shock. A small number of guinea pigs showed a mild form of tuberculin shock, in which the temperature showed an initial rise followed later by a moderate fall of usually not more than

Fig. 6



THE EFFECT OF THE DOSAGE OF PPD ON THE TYPE OF SYSTEMIC REACTION PRODUCED.
(a) guinea pigs infected 3 weeks (3 animals);(b) infected 8½ weeks(I animal)

2 to 3°F. below the preinjection value by the end of 8 hours after injection. The symptoms of shock were confined to dullness, inappetance and some elevation of the fur and were followed by a complete recovery in 24 hours except for a slight reduction in rectal temperature. (In one very mildly shocked guinea pig, the temperature was lower at 24 than at 8 hours ; this animal had completely recovered by 48 hours.)

Factors Determing the Type of Systemic Reaction

The type of systemic reaction to which an injection of PPD gives rise was found to be related to several factors.

Duration of infection. The length of time that the animal has been infected with tuberculosis has an important bearing on its response to an injection of PPD. In Fig. 5 are shown the temperature responses of 12 contemporaneously infected guinea pigs (dose : 0.001 mg. VPL), injected with 2 mg. of PPD at intervals varying from 2 to 12 weeks after infection. The type of response varied with the time interval.

At 2 weeks, there was no detectable temperature response. At 3 to 11 weeks, most of the animals showed simple febrile responses. At 9 to 12 weeks, most of the animals developed tuberculin shock. However, guinea pigs could be rendered susceptible to shock at an earlier stage by producing a more severe form of tuberculosis. In a small group of animals infected with 0.01 mg. tubercle bacilli (VPL), 2 mg. of PPD regularly produced shock after only 5 to 6 weeks infection.

TABLE 2

THE EFFECTS OF 2MG. PPD ON CIRCULATING LEUCOCYTES

Group	*Hours*	*0*	*1*			*4*			*6*			*24*		
		T.L.N.	T.	L.	N.	T.	L.	N.	T.	L.	N.	T.	L.	N.
Tuberculous, injected with 2mg. PPD.	Nos.	14	10	10	10	6	6	6	9	9	9	9	8	8
	Mean	100	92.24	67.01	135.75	32.25	32.97	29.17	55.35	36.22	92 91	84.61	74.29	115.36
	S.D.	—	70.44	33.31	164.02	17.44	21.38	11.75	29.32	24.63	59.75	38.81	45.60	83.45
Normal, injected with 2mg. PPD	Nos.	10	10	10	10	9	9	9	10	10	10	10	10	10
	Mean	100	84.58	71.78	98.12	114.02	80.98	125.92	88.85	65.16	111.90	102.92	108.39	99.39
	S.D.	—	18.40	21.87	30.52	54.69	35.28	82.33	33.47	20.27	50.73	33.45	54.02	31.14
Tuberculous guinea pigs, uninjected.	Nos.	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	100	110.12	106.88	111.60	80.60	74.16	90.20	111.54	106.60	123.84	82.32	100.46	77.36
	S.D.	—	41.67	46.48	41.39	21.36	32.39	32.42	35.13	25.16	73.41	28.85	42.35	43.58
Normal guinea pigs, uninjected	Nos.	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	100	109.48	95.34	126.90	110.48	103.36	119.80	113.40	95.58	129.82	98.42	96.26	108.56
	S.D.	—	16.82	28.03	32.45	38.24	33.33	48.13	41.06	41.66	48.34	12.97	30.77	37.72

All values are given in terms of the mean percentage of the respective initial values in the animals studied.

T—total count ; L—lymphocytes+monocytes ; N—neutrophils.

Significance of differences between mean values in tuberculous and normal guinea-pigs, when injected with 2 mg. PPD.

Hours—>	0	*1*			*4*			*6*			*24*		
	T.L.N.	T.	L.	N.	T.	L.	N.	T.	L.	N.	T.	L.	N.
Values of P	—	>0.1	>0.1	>0.1	<0.01	<0.05	<0.05	<0.05	<0.05	>0.1	>0.1	>0.1	>0.1

The dose of tuberculin. The relationship between the dose of tuberculin and the type of response that it produced was investigated at two different stages of infection. Three guinea pigs, infected 3 weeks (0.001 mg. IAP) and giving positive intradermal reactions, were injected subcutaneously with 2, 20, and 40 mg. of PPD respectively. With 2 mg., there was only a febrile reaction without shock in any form. With 20 mg. typical shock developed after a preliminary temperature rise. With 40 mg., there was severe shock which apparently developed earlier than after 20 mg. (Fig. 6) In a normal control guinea pig, 40 mg. of PPD produced no significant effect upon either the temperature curve or the clinical condition and the animal remained normal throughout.

A similarly infected guinea pig, 8½ weeks after inoculation, was then injected subcutaneously with 0.1 and 0.5 mg. of PPD on sucessive days. 0.1 mg. of PPD produced a mild but probably significant temperature rise subsiding after a few hours without evidence of shock, while 0.5 mg. the following day gave rise to fatal tuberculin shock. (Fig. 6).

Evidently, either the febrile or the shock type of response can be elicited at any stage of the disease, according to the doses of PPD employed, but the amounts required to produce either form of reaction decrease as infection progresses.

Relationship to Cutaneous Hypersensitivity

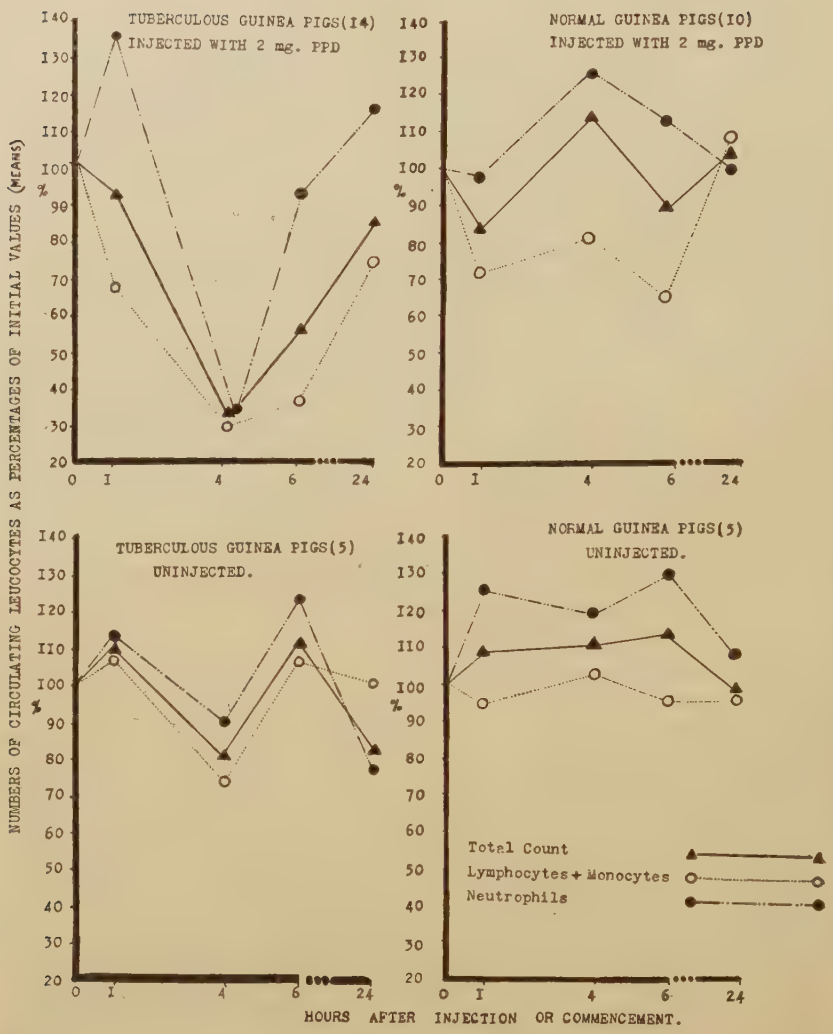
The degree of skin reaction elicited by a standard intradermal dose of PPD (320 i.u.) increased fairly regularly as infection progressed. In guinea pigs infected with 0.001 mg. of tubercle bacilli (VPL), the skin reactions from 6 weeks onwards were accompanied with increasing frequency by extensive necrosis and central haemmorhage. By the time these guinea pigs had become susceptible to shock with 2 mg. of PPD (about 9 weeks), the skin reactions were usually intense. However, using 2 mg. of PPD as the standard dose in the systemic test, shock could sometimes be elicited in animals giving only erythemic intradermal reactions, while other guinea pigs giving violent ' cocarde ' skin responses displayed only febrile reactions when tested systemically. Thus, there was not always a close relationship between the degree of skin hypersensitivity and the clinical form of the systemic reaction.

The Haematological Picture during the Systemic Tuberculin Reaction

The injection of tuberculin into normal guinea pigs gave rise to no significant alterations in the haematological picture, and the variations in the numbers of leucocytes and platelets in the course of serial observations probably represented normal physiological fluctuations. The injection of PPD into tuberculous guinea pigs, however produced several marked changes in the leucocyte picture.

Changes in the total white cell count. After the injection of 2 mg. of

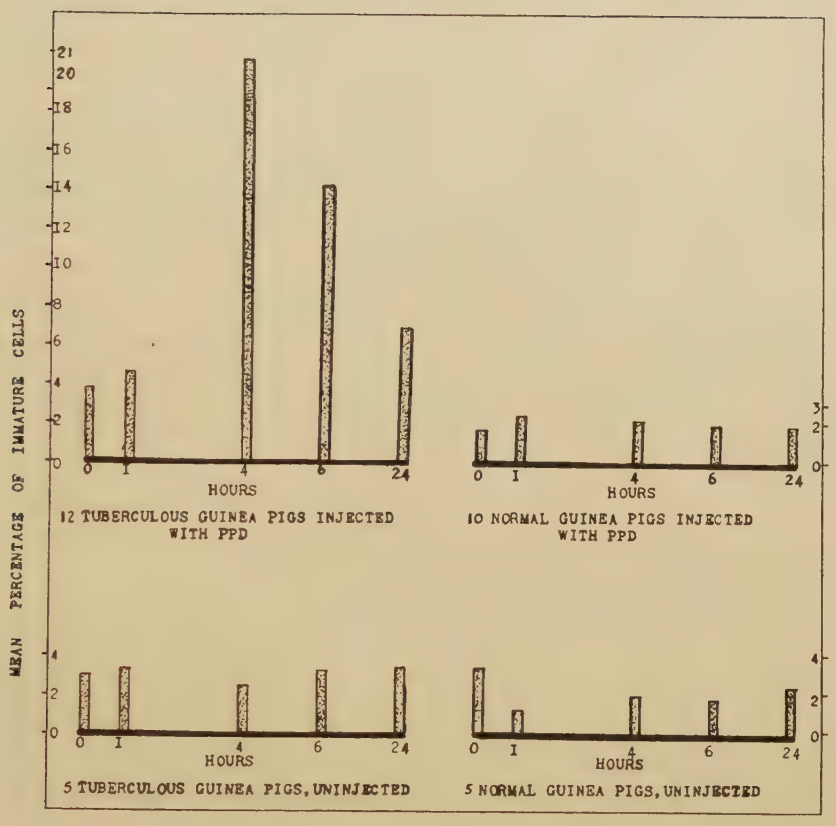
Fig. 7



PPD into tuberculous guinea pigs, the total leucocyte count fell after 4 hours to an average of about one third (32.25 per cent) of its pre-injection value. This leucopenia was only transitory ; it was occasionally evident as early as 1 hour after injection and by 6 hours it was tending to recede. The total leucocyte count had usually returned to within the normal range by 24 hours. (Fig. 7).

Changes in the numbers of neutrophils and non-granular leucocytes. During the phase of maximal leucopenia at 4 hours, both the neutrophils and the non-granular cells (i.e. the lymphocytes + monocytes) were considerably reduced in numbers, and the count in each group averages only about one third of its respective initial value. By 6

Fig. 8


MEAN PERCENTAGE OF IMMATURE CELLS
HOURS
12 TUBERCULOUS GUINEA PIGS INJECTED WITH PPD
10 NORMAL GUINEA PIGS INJECTED WITH PPD
5 TUBERCULOUS GUINEA PIGS, UNINJECTED
5 NORMAL GUINEA PIGS, UNINJECTED

PERCENTAGE OF NEUTROPHIL COUNT CONTRIBUTED BY 'BAND' AND OTHER IMMATURE FORMS.

hours, the mean neutrophil count had returned to normal, but the non-granular count remained low and was still somewhat reduced at 24 hours. (Fig. 7). At 4 hours all the mean values were significantly lower than in PPD-injected normal controls. At 6 hours however, only the total and the non-granular counts differed significantly. (Table 2). No increase in the numbers of eosinophils was observed at any stage of the systemic reaction.

Changes in the numbers of immature neutrophils. The average numbers of circulating immature neutrophils during the experimental period (expressed as percentages of all neutrophils) are shown in Fig. 8. In the PPD-injected tuberculous guinea pigs, but not in the control groups, there was a marked increase in the proportion of band and other immature neutrophils after 4 hours, and this persisted with declining intensity at 6 and 24 hours. Calculations of the actual numbers of immature neutrophils in the blood during the leucopenic

phase revealed that these cells, in contrast to the other leucocytes, usually showed an absolute numerical increase, which was sometimes evident as early as one hour after injection.

The Relationship of the Leucopenia to other Experimental Factors.

There was no apparent correlation between the degree of leucopenia which PPD produced in individual guinea pigs, and their cutaneous hypersensitivity or the duration of infection. Severe degrees of leucopenia were as frequently associated with reactions of the febrile type as with tuberculin shock.

The Platelet Count during the Systemic Tuberculin Reaction.

There were no consistent changes in the platelet count during the systemic tuberculin reaction in any of its forms. The platelet counts 24 hours after injecting tuberculin tended to be rather lower than in PPD-injected normal controls, but the difference was not statistically significant.

The Pathology of the Systemic Tuberculin Reaction

The Effects of Tuberculin on the Tissues of the Injection Site

When tuberculin was injected in 2 mg. doses into the muscles of the left thigh, it produced no visible local effects. However, when it was injected into the right thigh—the site of primary tuberculous infection—it often gave rise to an intense gelatinous oedema around the local tuberculous lymph nodes, extending from the lower part of the thigh to as far as the costal margin. (Very occasionally similar oedema occurred even after injection of tuberculin at sites distant from this area.) PPD, injected intraperitoneally, sometimes gave rise to mild peritonitis, but this effect was not constant.

The perifocal reactions. Focal haemorrhages in the spleen and liver, and less regularly, in the lungs and tuberculous lymph nodes, were a conspicuous feature of the autopsy picture in guinea pigs that had developed tuberculin shock but, as will be seen from Table 3 they were almost invariably absent in animals affected only with febrile reactions. In the spleen, these changes took the form of intense engorgement, sometimes associated with conspicuous red infarcts. Congestion was sometimes evident in the spleens of the uninjected tuberculous controls, but to a much less regular and severe degree than in the guinea pigs that had received PPD. In the liver and lungs, the focal reaction produced a strikingly florid picture with multiple bold haemorrhages in many of the areas of normal tissue separating the tuberculous lesions. In places, they had coalesced to form conspicuous haemmorhagic areas, while isolated tubercles sometimes showed typical perifocal 'flares.' In two animals, densely scattered ecchymoses were present throughout the liver even though hepatic tuberculosis was minimal. In the lungs, even when tuberculous involvement was severe, haemorrhagic

No.	Time infected (weeks)	Infecting dose (m.g.)	Dose of PPD (mg.)	Degree of shock	Haemorrhagic Lesions: Lymph nodes (R.H. leg)	Thigh muscles (R.H. leg)	Spleen	Liver	Lung	Skin test site.
				A. Guinea pigs displaying simple febrile reactions only						
747	3	0.001	2	o	o	o	o	o	o	o
701	4	,,	2	o	o	o	o	o	o	o
769	4	,,	2	o	o	o	*	o	o	o
30	5	,,	2	o	o	o	o	o	o	o
126	6	,,	2	o	o	o	**	o	o	o
921	7	,,	2	o	o	o	***	o	o	o
493	7	,,	2	o	o	o	***	o	o	o
171	8	,,	2	o	o	o	***	o	o	o
991	9	,,	2	o	o	o	****	*	o	o
375	11	,,	2	o	o	o	*	*	o	o
529	3	0.01	2	o	o	o	*	o	o	o
525	5	,,	2	o	o	o	o	o	o	o
319	3	0.1	2	o	o	o	o	o	o	o
				B. Guinea pigs displaying tuberculin shock						
553	6	0.001 i/p	2	*	–	–	**	o	o	o
263	6	0.01	2	*	o	o	**	o	o	***
934	8	0.001	2	*	o	o	**	o	o	o
156	12	10.0 (BCG)	2	*	–	–	–	–	–	*
944	5	0.001	2	**	P.Pr.S.	****	***	o	o	****
449	6	,,	2	**	o	o	***	o	o	****
437	7	,,	1	**	o	****	***	***	o	–
862	6	,,	1	***	P.	****	**	***	o	–
1155	3	,, (IAP)	20	***	P.	o	o	o	o	*
283	3	,, (IAP)	40	***	P.Pr.	o	***	***	o	****
464	5	0.01	1	***	Pr.I.P.	****	****	***	o	–
115	7	,,	1	***	S.	o	****	****	*	–
130	7	,,	1.8†	***	P.Pr.	****	****	****	****	–
803	9	0.001	2	***	o	****	****	***	o	****
673	10	,,	2	****	P.	o	***	**	o	****
151	11	,,	2	**** (died 7 hour).	P.Pr.I.	****	***	****	o	–
914	5	0.001 i/p	2	**** (died 8 hour).	–	–	**	*	o	****
342	8	0.02	0l8	**** (died 6 hour.)	P.	o	****	****	o	–
727	16	500 (killed) i/p	2	**** (died 5 hour.)	–	–	*	****	o	–
453	8	0.001	0.4	**** (died 5 hour.)	o	o	****	**	o	–
543	7	,,	2	**** (died 4 hour.)	P.	o	****	*	o	–

Notes 1. *Infecting dose.* Except where otherwise stated, the guinea pigs were infected with bovine tubercle bacilli (strains VPL or 3076).
2. *Lymph Nodes.* The congested lymph nodes were indicated as follows : P—popliteal ; Pr.—precrural; S—sublumbar ; I—inguinal.
3. *Haemorrhagic Lesions.* *, **, ***, ****, indicate increasing degrees of haemorrhagic and congestive change.
† The tuberculin was injected into the right (tuberculous) thigh in this guinea pig.

reactions were relatively uncommon. In the tuberculous lymph nodes, the reaction took the form of capsular congestion. Over the inner aspect of the right thigh, at the site of primary inoculation, there was often an intense haemorrhagic change, apparently related to the presence of small tubercles scattered throughout the local muscular tissue.

Many tuberculin shocked guinea pigs (9 out of 14) displayed adrenal haemorrhagic changes in the form of cortical petechiae, congestion of the cortex, or less frequently, the medulla. Cortical petechiae were found in 1 guinea pig out of 7 examined after simple febrile reactions.

Pulmonary emphysema, bronchospasm and congestion of the alimentary canal were all absent. Subcutaneous dehydration was sometimes present but its incidence was irregular.

Microscopical changes. The focal reaction consisted of intense engorgement of the local capillaries with erythrocytes, which tightly plugged the vessels at the periphery of the tuberculous lesions. In places, the capillary wall had disappeared and the contained cells had been shed into the surrounding tissues. There was no evidence that the systemic reaction gave rise to local inflammation or leucocytic infiltration around the tubercles. In the guinea pigs that had sustained febrile systemic reactions, (but also in the tuberculous controls) thrombi of platelets with entangled leucocytes were occasionally found in the veins of the tuberculous spleen and liver. The majority of the shocked guinea pigs, however, showed thrombi at these sites, and occasionally in other organs such as venules of the kidney and tuberculous lymph nodes. The frequency of these changes in the shocked guinea pigs is possibly a result of disturbed circulatory conditions, rather than any specific effect of tuberculin.

In the adrenal glands, the zona reticularis sometimes showed areas of cells with nuclear pyknosis and staining anomalies, but whether the systemic reaction played any part in their production is uncertain, since adrenal degenerative changes of this type are a feature of tuberculosis. However, the single guinea pig displaying adrenal haemorrhages after a febrile systemic reaction showed a focus of actue cortical necrosis, with disappearance of the local parenchymatous cells, neutrophil infiltration, and regenerative hyperplasia in the surrounding tissue. The recency of this lesion suggested a connection with the systemic reaction which the animal had sustained. The adrenal vascular changes consisted of focal or diffuse sinusoidal congestion.

One shocked guinea pig had a marked peribronchial eosinophilia ; eosinophils were also present in the interstitial tissue of the kidneys and myocardium, and in the small myocardial blood vessels in this animal.

Experiments on the Role of Histamine in Tuberculin Shock

Although there are many striking differences between the manifestations of delayed hypersensitivity and anaphylaxis, both conditions probably arise from the union of antigen with cellular antibody. In anaphylaxis, release of histamine and other substances has been amply demonstrated by many workers to be a consequence of this antigen-antibody reaction. It seems possible that some of the severe physiological disturbances associated with tuberculin shock might similarly be the result of the release from the cells of some active pharmacological agent. The experiments to be described represent a preliminary exploration of this posibility. The present inquiry, however, is confined to the role of histamine in this connection.

The Blood Histamine Levels in Guinea Pigs with Tuberculin Shock

The concentration of histamine in the blood of guinea pigs with tuberculin shock was compared with that of tuberculous and normal controls (Table 4). All the guinea pigs with tuberculosis had been infected for at least 28 days and had well established lesions of the disease.

TABLE 4

Blood Histamine Levels

Group	*No. of animals*	*Mean blood histamine concn. μg. histamine base /ml.*	
Normal	10 (4M 6 F)	0.117	± 0.050
Tuberculous	10 (2M 8 F)	0.142	± 0.078
Tuberculin Shocked	10 (5M 5 F)	0.088	± 0.029

There was no evidence of the entry of histamine into the blood in detectable quantities, in contrast to the situation in anaphylaxis in the guinea pig (Code, 1939). The values in tuberculin shock were, indeed, lower than in the tuberculous controls, although the difference is not significant statistically.

The Perfusion of the Isolated Lungs of Tuberculous Guinea Pigs with Solutions of Tuberculin

Although the results of the blood histamine studies did not support the suggestion that histamine might be released during tuberculin shock, nevertheless, it seemed desirable to supplement those observations by others of a more sensitive character. In the whole blood, only a proportion of histamine is present in an active form, so that variations in the amount of the active fraction might be masked by those of the larger inactive component.

The isolated lungs of tuberculous guinea pigs were therefore perfused with solutions of PPD in Tyrode's Solution, so that any liberated histamine might be detected in the venous perfusate. The guinea pigs used in these experiments had reached a stage of tuber-

TABLE 5

RELEASE OF HISTAMINE FROM THE LUNGS OF TUBERCULOUS GUINEA-PIGS

No.	*Sex*	*Weight (gm.)*	*Perfusion conditions ; concentration of PPD in the Tyrode's Solution.*	*Total PPD passing through the lungs (mg.)*	*Histamine (u.g.)*	
					Released on perfusion	*Remaining in the lung*
			TUBERCULOUS GUINEA-PIGS			
582	M	315	Perfused 4 hours with Tyrode containing 8 mgs. PPD/litre	3.6	0.44	55.4
51	F	340	As above	3.6	0.77	9.1
632	M	320	Perfused 6 hours ; 100 mgs. PPD/litre of Tyrode	22.3	0.14	25.4
271	F	278	Perfused 7 hours ; 100 mgs. PPD/litre of Tyrode	50.4	0.43	44.0
123	M	—	Perfused 8 hours ; 100 mgs. PPD/litre of Tyrode	61.9	0.14	63.8
575	F	447	Perfused 2 hours with Tyrode containing 100 mgs. PPD/litre, then 2 hours with Tyrode only	9.8	1.43	66.7
92	F	371	10 mgs. PPD injected directly through cannula then perfused 5 hours with Tyrode.	10.0	0.30	50.0
246	F	397	As above	10.0	0.47	46.7
			NORMAL GUINEA-PIGS			
1.	M	458	Perfused 4 hours ; 8 mgs. PPD/litre of Tyrode	2.2	0.20	80.0
2.	F	388	Perfused 4 hours ; „ „ „	3.7	0.70	47.9
3.	M	295	Perfused 8 hours ; „ „ „	3.1	0.69	61.1
4.	F	278	Perfused 6 hours ; „ „ „	3.5	0.78	22.1

culosis at which the intramuscular injection of 2 mg. of PPD regularly gave rise to tuberculin shock. None of the animals used was anaphylactically sensitive ; a strip of plain muscle from each guinea pig was suspended in a bath and tested by the addition of 1 to 2 mg. of PPD, but no contractions were recorded.

It is evident from the findings in Table 5 that, under the experimental conditions described, tuberculin is not able to effect a release of histamine from the tissues of the hypersensitive animal. The trivial amounts present in the perfusion fluid after it had traversed the lungs were quantitatively similar in both the tuberculous and normal guinea pigs.

DISCUSSION

In the normal guinea pig, PPD was quite innocuous, but when it was injected into a tuberculous animal, it gave rise to a marked constitutional reaction which assumed one of two different forms. There was either a simple febrile reaction, which disappeared after a few hours leaving the animal quite normal again, or alternatively, tuberculin shock developed. In guinea pigs with progressive tuberculosis, the type of reaction which a standard dose of PPD (2 mg.) produced was found to be contingent upon the stage of the disease at which it was administered. In the early stages it produced a febrile type of response, but as the disease advanced, the same dose of PPD usually gave rise to tuberculin shock. It has been shown that as the disease progressed the threshhold dose of PPD required to produce either type of reaction decreased. There would thus appear to be a reciprocal relationship between the dose of PPD required to produce shock, and the stage of the disease at which it is administered.

In accounting for the changing response to a constant dose of PPD during the course of the infection, it is difficult to believe that the action of the PPD on the tuberculous body differs in the two forms of the systemic reaction, and it seems more likely that the differing clinical pictures reflect quantitative differences in the intensity of its action on the body. The characters of both forms of the systemic reaction are consistent with the general view that PPD specifically damages the cells of the tuberculous body. In its capacity to induce a febrile reaction, PPD resembles the pyrogenic endotoxins produced by many bacteria, but differs from them in its specificity. It also resembles them in that a short lag period elapses before its pyrexial effect becomes apparent. Beeston (1947) has suggested that fever, whether produced by bacterial pyrogens or other agencies, results from damage to tissue cells and release therefrom of diffusible substance into the blood stream having a pyrogenic effect on the heat regulatory centre in the hypothalamus. There is further evidence that in the systemic reaction PPD produces cellular damage in the outpouring of immature neutrophils into the circulation which follows the injection of tuberculin. Tuberculin shock therefore,

probably results from a similar form of cellular injury to that seen in the febrile reaction, but on a more intense and widespread scale. Although tuberculin shock was usually accompanied by multiple focal haemorrhages, the present evidence does not support the view that these are the immediate causes of shock, since, in some instances, there was very severe shock even though the haemorrhagic changes were minimal. However, when widespread and numerous perifocal reactions are present, they would doubtless tend to exacerbate the state of shock.

It is a finding of some interest that PPL, injected intramuscularly, produces detectable clinical effects in the form of a rise in temperature as early as 1 hour after its introduction and may kill the animal in 4 hours, while the intradermal response to PPD does not usually become apparent for some 10 to 15 hours. This relatively rapid action of PPD lends support to the conclusion reached by Swithinbank, Vollum and Smith (1953), on the basis of their studies of the intrathecal tuberculin reaction in man, that tuberculin becomes ' fixed ' to the cells at its site of action at an early stage after its introduction —probably within a few minutes.

The present work throws no light upon the role which antibody plays in the manifestations of tuberculin hypersensitivity. The evidence in favour of the participation of an antibody in delayed hypersensitivity is very strong, and, indeed, it is difficult to explain the high degree of specificity exemplified in the tuberculin reaction on any other basis than that of an antigen-antibody phenomenon. If it is accepted that the cells of the tuberculous animal become ' sensitised ' to the action of PPD in consequence of their acquisition of specific antibody, it is tempting to attribute the increasingly toxic effects of PPD as tuberculosis advances to an increase in the amount of cellular antibody and a correlative enhancement of the capacity of the cells to take up and ' fix ' the administered antigen. However, it is possible that other factors besides antigen and antibody play a part in the systemic reaction, and Shwartzman (1937), in particular, has emphasised the possibility that non-specific ' active principles ' in the blood of tuberculous animals might be important reagents in the manifestations of hypersensitivity.

Study of the pharmacological aspects of tuberculin hypersensitivity was confined to the role of histamine, although clearly, there are several pharmacologically active substances whose activities in this connection might be worth exploring. The attempts to demonstrate a release of histamine from the cells under the influence of PPD gave only negative results, even though in the perfusion experiments, the isolated lungs were exposed to amounts of PPD 25 to 30 times as great as that required to produce tuberculin shock in vivo. This would suggest that histamine release is not an important factor in tuberculin hypersensitivity—a conclusion already indicated by studies of the effects of antihistamine drugs on the tuberculin

reaction (Weiser, Evans and St. Vincent, 1950 ; Swithinbank, Smith and Vollum, 1953). However, the possibility remains that the use of blood as the perfusion fluid instead of Tyrode's Solution is necessary in order to provide the conditions for PPD to act on the cells.

CONCLUSIONS

The systemic tuberculin reaction in the guinea pig may assume either of two principal forms :— (a) a transitory febrile reaction which disappears after some hours, leaving the animal quite normal again ; (b) tuberculin shock characterised either by a preliminary temperature rise, followed by falling temperature, circulatory collapse and death 4 to 30 hours after injecting tuberculin or in occasional animals a very mild non-fatal form of tuberculin shock.

When a standard dose of PPD is injected into tuberculous guinea pigs in various stages of tuberculosis, it gives rise to the febrile type of response early in the disease and tuberculin shock in the later stages. The dose of PPD necessary to produce shock decreases as infection progresses.

PPD in tuberculous guinea pigs causes a marked leucopenia, most intense 4 hours after injection ; the lymphocytes and monocytes remain depleted in numbers for rather longer than the neutrophils. PPD also caused a considerable outpouring of immature neutrophils into the circulation within 1 to 4 hours of injecting it.

At autopsy, focal and confluent haemmorhages and congestion in the liver, spleen and sometimes other organs are characteristic of the shock type of systemic reaction. They are usually absent after febrile responses. Haemorrhagic changes in the adrenals occur after both forms of reaction but more often after tuberculin shock.

It is suggested that both the febrile and the shock forms of reaction are clinical expressions of cellular damage, differing quantitatively in intensity.

It is not possible to demonstrate a release of histamine from the isolated lungs of tuberculous guinea pigs when perfused with solutions of PPD.

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EXPERIMENTAL STREPTOCOCCAL MASTITIS

IX. The Disease in the Cow

By

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INTRODUCTION

In the previous work recorded in this series of papers the goat was used as the experimental animal for reasons of economy and convenience. Although many of the observations made in goats appeared to be applicable to the cow, it was necessary to confirm some of these findings by the experimental inoculation of cows. The work carried out in goats had revealed no major difficulty in establishing infection by direct inoculation into the teat sinus, but the literature, as summarised by Plastridge (1953), indicates that the same procedure carried out in the cow meets with varying success.

This paper records the observations made following introduction of one or other of two strains of *Streptococcus agalactiae* into the teat sinuses of 35 cows.

MATERIALS AND METHODS

Experimental animals. First-and second-calf Ayrshire cows continuously housed in the isolation units at this Field Station were used. All the cows had been exposed to infection with *Brucella abortus* and a minority of them had aborted. *Str. agalactiae* had not been recovered from any cow in these isolation units, but clinical cases of staphylococcal mastitis had occurred. Prior to inoculation with *Str. agalactiae* the milks of all the cows were subjected to cultural examination, Whiteside test and neutrophil count (*vide infra*) and the quarters to be inoculated were negative to these tests. There was no reason to believe that their previous history would influence their usefulness for our studies.

Strains of Str. agalactiae Strain S 13 Lancefield group B type Ib, as used in the studies on goats, was used in one series of observations, and strain A100, Lancefield group B type II in a second series. Strain S 13 was possibly of human origin (Pattison and Holman, 1953), but strain A100 was a bovine strain.

Infecting dose. This consisted of an 18-hour Todd-Hewitt broth culture diluted to give the required number of organisms in a volume of 10 ml. using Todd-Hewitt broth as the diluent. One quarter only of each cow was inoculated, and the appropriate dilution was introduced, within 30 minutes of preparation, into the teat sinus by means of a syringe and smooth-ended 18 S.W.G. needle. A viable count was carried out on the residual inoculum ; counts of strain S 13 were made by the plate method without shaking the culture, and results (Table I) are given as powers of ten ; counts of A 100 were made by the surface drop method after shaking the culture for about three minutes, and results (Table 3) are given as five times powers of ten. Inoculation took place approximately three hours after the morning milking and the cows were milked out as usual in

the early afternoon ; the interval between inoculation and afternoon milking was therefore about five hours.

Clinical examination. Udders were examined and temperatures recorded twice daily. During periods of systemic disturbance more frequent examinations were made, and in the later phases of the work, when it became apparent that febrile reactions played an important part in the disease process, temperatures were recorded every six hours throughout the day and night.

Milking and milk samples. Milking was carried out with a Gascoigne individual quarter machine (Hall, 1951). To obviate spread of infection during milking a fresh sterile liner was used for each inoculated quarter. All samples taken for laboratory examination were obtained from the fore-milk.

Bacteriological examination of the milk. The fore-milk was examined culturally and an approximate viable count was made twice daily (Pattison, Taylor and Holman, 1950). Colonies of strain S13 were readily countable after 18 hours incubation, but on occasion strain A100 required 36 hours. Every two or three days the organisms recovered from the milk were identified serologically. Samples from the three uninoculated quarters were examined once weekly in order to detect any spread of infection to other quarters. With one group of six cows, inoculated with strain S 13, particular attention was paid to the apparent disappearance of the inoculated streptococcus from five of them. Attempts were made to demonstrate the organisms by a concentration method, as follows :— All the milk from the inoculated quarter was spun down and cultures were made from the gravity cream and from the deposit into crystal violet broth and on to Edwards medium (Edwards, 1933) ; the crystal violet broth was used as an enrichment medium and such growth as was obtained in it was plated on to Bactotryptose ox-blood agar.

Macroscopic appearance of the milk. A daily record was made of the macroscopic appearance of the milk from the inoculated quarter and a figure was given to each sample as follows :— 0 Normal colour and consistency, 1 Normal colour with a fine sediment, 2 Normal colour with fine flakes, 3 Normal or greyish colour with large flakes, 4 Normal or greyish colour with clots, 5 Yellowish or watery with large mucoid clots.

Milk yields. Considerable importance was attached to the loss of milk in the inoculated quarters as a measure of the intensity of the mastitis provoked. Before infection, one quarter of each cow was selected to be infected and another to act as the control; quarters giving similar amounts of milk were used. The total yield of each of these quarters was measured for one week before infection, and after infection the yield of the control quarter was accepted as the standard from which the yield of the infected quarter could be determined.

Supplementary tests for mastitis. The following tests for mastitis were also carried out in order to ascertain their relative value ; they were not all employed in all experiments.

Neutrophil count. The modified Breed smear technique (Pattison and Holman, 1951) in which only neutrophils were counted was employed.

Chlorides. These were estimated either by titration or by a Rapid Abnormality Indicator.

TABLE I

INFECTION OF COWS WITH *Str. agalactiae* STRAIN S 13

Experiment no.	*Cow No.*	*No. of viable organisms*	*Whether infection established*	*Type of syndrome established*	*No. of days between inoculation and first crisis*	*Recordings continued for (days)*
1	1	10^9 (dead organisms)	no	—	*	9
	2	10^9	doubtful	transient	*	,,
	3	10^7	doubtful	transient	*	,,
	4	10^5	no	—	*	,,
	5	10^3	yes	prolonged?	4	,,
2	6	10^9	doubtful	transient	*	108
	7	10^9	doubtful	transient	*	,,
	8	10^9	doubtful	transient	*	,,
	9	10^9	yes	persistent	No crisis recorded	,,
	10	10^9	yes	prolonged	7	,,
	11	10^9	doubtful	transient	*	,,

* no crises as infection not established.

Whiteside test. The degree of coagulation caused by mixing two drops of milk with one drop of N/1 NaOH on a glass tile was recorded as 0, 1, 2, 3, or 4.

pH. This was estimated by means of a pH-meter.

Sugar reduction. The amount of quantitative Benedict's solution reduced by 1 ml. of milk was calculated from the amount of milk which would reduce 10 ml. of Benedict's solution.

Serum agglutination test. This was carried out as described by Holman, Pattison, and Gordon (1952), using the two strains of *Str. agalactiae* as antigens.

Attempts to provoke a clinical mastitis following disappearance of the organisms from routine samples. Histological studies in the experimentally infected goat (Pattison, 1951) had revealed the blocking of ducts by granulation tissue in infected udders, and it was possible that *Str. agalactiae* although absent from the milk might exist above these lesions that are known to occur also in cows. It was decided, therefore, to attempt to break down these lesions in some cows, by subjecting the quarter to trauma or by injecting into the udder testicular hyaluronidase (Evans). Trauma was caused by injecting directly into the galactiferous sinus, by means of a Higginson's pump and teat syphon, four to six litres of sterile distilled water at about 2°C. The second procedure involved the injection of 30 mg. of hyaluronidase in 10 ml. of sterile distilled water.

These procedures were carried out on the group of five cows referred to in paragraph 6 above.

Post-mortem bacteriological examination. The inoculated quarters of these same five cows were removed immediately after slaughter, and, using sterile precautions, a piece of tissue about 10 cm. in diameter was removed from the centre of the quarter and macerated in an electric mincer and then transferred to three litres of Hartley broth. After overnight incubation at 37°C. three samples from each were plated on to Bactotryptose ox-blood agar. In the case of these animals, and some others also, cultures were made from the supra-mammary lymph nodes.

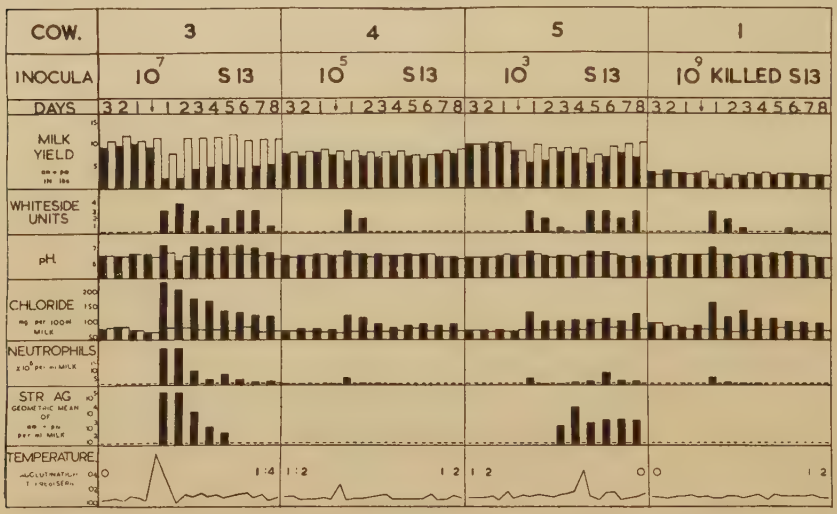
RESULTS

EXPERIMENTS USING STRAIN S 13

Graded Doses of the Infecting Organism

Five cows were used in this experiment, four of which were inoculated into one quarter with respectively 10^9, 10^7, 10^5, and 10^3 viable organisms, and one with 10^9 heat-killed organisms. The animals were slaughtered after nine days.

Fig. 1.



Reaction of four cows over 9 days following the injection of different numbers of S 13. Black columns represent results for the inoculated quarter ; white column for the control quarter. When the measurement was zero the result is shown as a dash. Agglutination titres are those before inoculation and on the ninth day.

The results of the various measurements made on these animals are shown in Table I and Fig. 1. Only four cows are included in the latter because the reactions of aimals 2 and 3 (10^9 and 10^7 viable organisms respectively) were closely similar. It will be seen that the reactions of the animals inoculated with living organisms were quite different from each other.

Cows 2 and 3. In these two cases living organisms, recovered during the first five days after inoculation, disappeared by the sixth day. The host response as a result of inoculating the infecting dose was marked in each case, as demonstrated by a febrile reaction,

a sustained drop in milk yield, a neutrophil response and abnormal chloride and Whiteside measurements. Evidently, however, although a mastitis was provoked, the disease was of short duration and was associated with failure of the organisms to become established in the mammary gland.

Cow 4. In this cow only slight changes were detected and living organisms were not recovered at any stage.

Cow 5. In this case the initial reaction was again slight and comparable to that seen in cow 4. On the third day after inoculation, however, organisms were recovered in large numbers, and this was associated with a febrile reaction, a marked neutrophil response, and a fall in milk yield. This gave the typical picture of a crisis as described in goats by Holman, *et al* (1952) and was similar to that mentioned by Jones and Little (1934). Cultural examinations of the supramammary lymph nodes of these cows were negative. The results for the supplementary tests for mastitis will be commented on later.

Changes Produced over a longer Period by Strain S 13

In the first experiment described above two cows appeared to throw off infection within a few days, but in another cow five negative cultural tests occurred between inoculation and the first milk sample in which *Str. agalactiae* was demonstrated. The fact that this latter cow gave negative samples during a period when *Str. agalactiae* was undoubtedly within the udder, made it necessary to find whether the two cows which yielded negative samples following a number of positive samples had in fact thrown off the infection, or whether it was still present in a concealed or quiescent form.

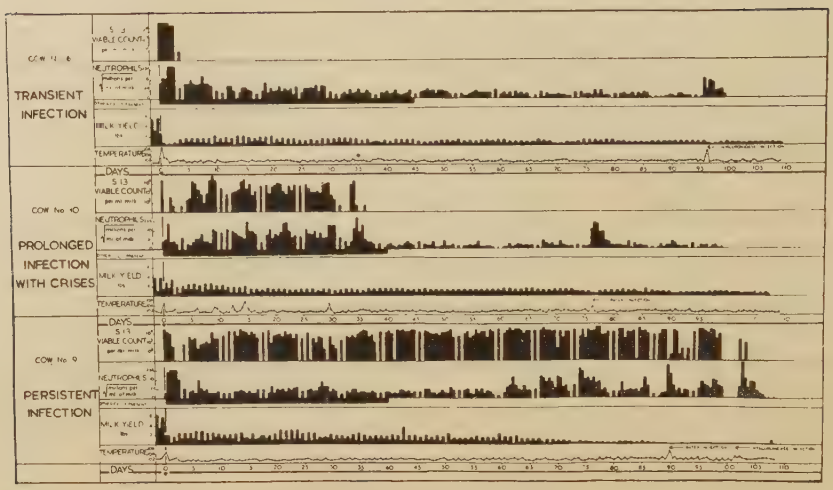
A more prolonged experiment was accordingly planned involving a further six animals, and as the two cows that appeared to throw off infection in Experiment I had received the two largest inoculations in the series, an infecting dose of 10^9 viable organisms was chosen. Six cows were inoculated with this dose and the experiment was continued for a period of 108 days, during which time the special methods described in the section headed " Materials and Methods " were variously applied in an effort to demonstrate streptococci in those cows in which they could no longer be recovered by routine sampling.

TABLE 2

INJECTION OF FLUID INTO QUARTERS PREVIOUSLY INOCULATED WITH S 13

	Day of experiment on which injection was made					
Cow No.	6	7	8	9	10	11
Cold water		69	77	91	76	
Testicular hyaluronidase ..	96			103		96

Fig. 2.



Different types of reaction over 108 days following inoculation with S 13. Negative measurements are shown as dots but these have been omitted for bacterial counts following the end of infection. Milk yield is that for the infected quarter only.

The results are given in Table I and Fig. 2 The type of reaction varied among individuals and has been classified, for descriptive purposes, as (a) transient (b) prolonged with crises (c) persistent.

Transient reaction. Three animals 6, 7 and 8, reacted with a transient reaction, which is depicted for cow 6 in Fig. 2. Streptococci disappeared from the milk within eight days of inoculation, and the reaction was characterized by a sharp and sustained loss of milk, abnormal neutrophil counts throughout the experiment, and the absence of crises. The reaction in cow 11 was also of the transient type in that streptococci were not isolated after the 16th day ; in this case, however, there was a mid-period of four days during which no organisms were recovered. The concentration method was applied to the milk of cows 6, 7 and 8 once weekly for six consecutive weeks from the 26th to the 68th day, with negative results. In addition, cows 7 and 8 received cold water traumatization and cow 6 received an injection of testicular hyaluronidase (Table 2). Both these treatments produced mastitis with abnormal milk and increased neutrophil count, but no organisms were recovered either from the milk or from the udder tissue which was cultured when the cows were slaughtered. It is doubtful whether the introduction of the inoculating dose into the udder of these cows really succeeded in establishing infection. This type of response was similar to that seen in cows 2 and 3 in the first experiment.

Prolonged infection with crises. In cow 10 (Fig. 2) organisms were recovered from some but not all samples taken during the first four days, but after that time they were constantly present in varying numbers up to the 30th day ; they finally disappeared altogether after the 37th day. From the 7th to the 30th day several typical crises occurred. Loss of milk was less than in the transient type of infection. The concentration method, cold water traumatization and cultural examination post mortem, failed to demonstrate the presence of streptococci after their disappearance from routine samples. The reaction in cow 5 in the first experiment may have been similar to that seen in this cow.

Persistent infection. In cow 9 (Fig 2) the most notable feature was the continued presence of organisms for the 90-day period that preceded traumatization of the udder. Neutrophil counts were consistently high but no crisis was observed. This cow was subjected to cold water traumatization and hyaluronidase injection to find out if these treatments had an effect on the excretion of the streptococci, but organisms continued to be recovered from succeeding samples.

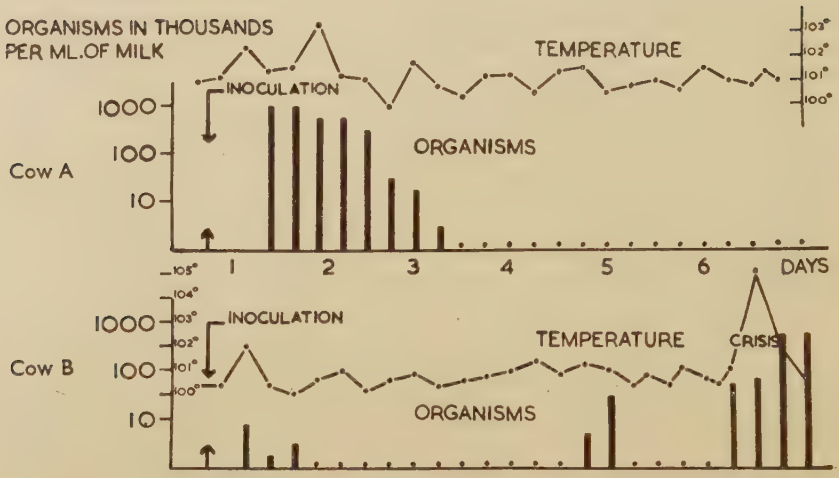
Cellular Content of Milk.

During the first two months of this experiment the presence of cells other than neutrophils in Breed smears occurred in considerable numbers in all animals for some four to six weeks after infection. The periods during which they were noted in the milk of cow 6, 9 and 10 are shown in Fig. 2, but their disappearance was gradual rather than sudden as might be inferred from the abrupt termination of the line in the figure. Pattison found that the pathological process in the goat was one of fibrosis and involution associated with an abnormal cell content of the milk and it is thought likely that the presence in these cows of cells other than neutrophils was associated with an involution process.

Macroscopic Appearance of the Milk from Infected Quarters

In transient infections gross abnormality (grades 3, 4 and 5) was usual for about five days, followed by a gradual return to normality in one to three weeks ; the milk was often obviously abnormal when *Str. agalactiae* could no longer be isolated. The persisent infection was characterised by the presence of flakes or clots (grades 2, 3 and 4) for about two days after inoculation, with a return to normal appearance by the end of one week ; thereafter *Str. agalactiae* was present in milk of normal appearance. In the cases of prolonged infection changes in the milk immediately after inoculation were of grades 1 or 2 and this degree of abnormality persisted over the period in which *Str. agalactiae* could be isolated from the milk. During crises abnormality of the milk was much more marked (grades 3 or 4). From these observations it was concluded that the macroscopic appearance of the milk cannot be relied upon to give an indication of its bacteriological status.

Fig. 3



Detection of streptococci in the milk of a transient reaction (cow A) and of a crisis infection (cow B). Note the eleven negative tests before the re-detection of streptococci in cow B.

Contrast of Transient Reaction with an Infection Producing Crises

Some idea of the fundamental difference between the transient type of reaction and the reaction which includes crises can be obtained from Fig. 3, which depicts the body temperature and the numbers of organisms recovered from milk samples from two cows, not included in the experiment described, which were sampled every six hours. Both cows received 10^9 viable strain S13. It will be seen that in cow A the organisms were not recovered after the third day, whereas in cow B very few were recovered initially and it was not until the sixth day that they became constantly present and gave a clear-cut picture of a crisis. The occurrence of 11 consecutive negative samples taken at six-hour intervals over three days is most striking and raises considerable speculation as to the location of the organisms during this time.

EXPERIMENTS USING STRAIN A100

Graded Doses of the Infecting Organism

Infecting doses ranging from a theoretical count of 5 to 5 x 10^8 viable organisms were inoculated into 24 cows as shown in Table 3. Four cows received an inoculum of 5 x 10^8, four 5 x 10^4 and four 5 x 10 ; two cows were inoculated with each of the other doses. The experiment carried out with the 5 x 10^8 inoculum preceded that in which the smaller doses were employed and recordings were continued in the case of cows 13, 14 and 15 for 46 days to see whether a typical crisis would develop. It is to be noted that surface drop counts made after inoculation of the six cows receiving the two smallest doses were negative.

TABLE 3

INFECTION OF COWS WITH *Str. agalactiae* STRAIN A100

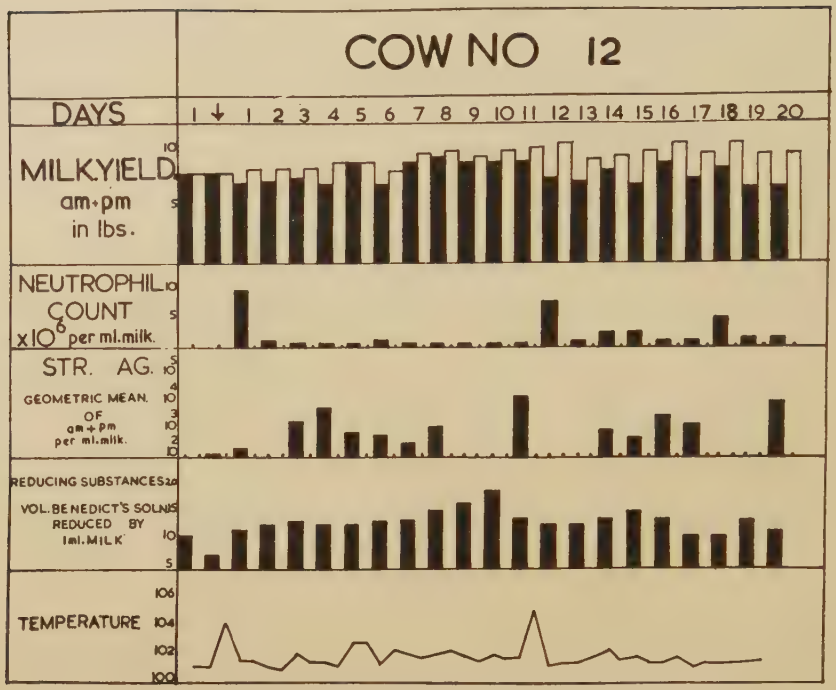
Cow No.	*Approx No. of viable organisms inoculated*	*Whether infection established* *	*No. of days between inoculation and first crisis*	*Recordings continued for (days)*
12	5×10^8	yes	11	20
13	5×10^8	yes	No crisis recorded	46
14	5×10^8	yes	No crisis recorded	,,
15	5×10^8	yes	No crisis recorded	,,
16	5×10^7	yes	4	10
17	5×10^7	yes	7	,,
18	5×10^6	yes	3	,,
19	5×10^6	no	—**	,,
20	5×10^5	yes	3	,,
21	5×10^5	no	—	,,
22	5×10^4	yes	2	,,
23	5×10^4	no	—	,,
24	5×10^4	no	—	,,
25	5×10^4	yes	2	,,
26	5×10^3	no	—	,,
27	5×10^3	no	—	,,
28	5×10^2	no	—	,,
29	5×10^2	no	—	,,
30	5×10	no	—	,,
31	5×10	no	—	,,
32	5×10	no	—	,,
33	5×10	no	—	,,
34	5	no	—	,,
35	5	no	—	,,

Notes :— * In those cows in which infection was established it was still present at the end of the experimental period.

** No crisis as infection not established.

The general results are shown in Table 3 and the crisis picture seen in cow 12 is shown in Fig. 4. It will be noted that infection was established in all four cows recieving the heaviest inoculum and in both cows receiving 5×10^7, but only in one out of two in the case of the 5×10^5 and 5×10^6 doses, and two of four given the 5×10^4 dose. A dose smaller than 5×10^4 viable organisms failed to infect. This apparent dependence of establishment of infection on the number of organisms injected confirms a similar observation made by Little (1937). The transient type of reaction noted with strain S 13 was not observed. Crises were recorded in all cows in which infection was established except in three out of the four receiving the largest dose. With the seven cows in which crises were recorded there was an initial period of from one to three days in which no organisms, or very few were recovered at routine sampling (about 1000 per ml. of milk.) This may be a manifestation of an incubation period.

Fig. 4


COW NO 12
DAYS
1 ↓ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
MILK YIELD am+pm in lbs.
NEUTROPHIL COUNT x10⁶ per ml. milk.
STR. AG. GEOMETRIC MEAN OF am+pm per ml. milk.
REDUCING SUBSTANCES VOL. BENEDICT'S SOLN REDUCED BY 1 ml. MILK
TEMPERATURE
106
104
102
100

A crisis produced by intramammary inoculation of *Str. agalactiae* strain A100 into cow No. 12.

With cows 17, 18, 20, 22 and 25 the return of organisms in larger numbers (10,000 or more per ml. of milk) was followed within 24 hours by a typical crisis.

Comparison of Results with Strains S 13 and A 100

The results obtained using the various infecting doses are tabulated in Table 4. The criterion of infection has been taken as the establishment of organisms within the udder and for this reason the transient type of reaction, in which the numbers of organisms recovered rapidly diminished following inoculation, has not been included as a valid infection.

Minimal infecting dose. We are not in a position to say what is the minimal infecting dose with strain S 13 as infection was established with the smallest dose employed. With strain A100, however, infection was not established below a dose of 5×10^4 viable organisms, as judged by the failure to infect ten cows which received a smaller dose than this.

Failure to infect. From the results given by cows 18 to 25 (Table 3) it is clear that failure to infect is not dependent only on the size of the inoculum ; it may be related to the strain of organism used, and to individual resistence on the part of the cow.

Crises. Typical crises were observed in all cases where infection was established except in the largest doses of each of the two strains.

Duration of infection. As a general rule the cows used in this series of experiments were disposed of at the end of the experimental period. However, an opportunity was taken to study the duration of infection in three cows infected early in lactation. Two were infected with strain A100 and one with strain S13. At the time of writing, nine months after inoculation, all three continue to shed *Str. agalactiae* in the milk of the infected quarter.

TABLE 4

COMPARISON OF RESULTS OBTAINED WITH STRAIN S13 AND STRAIN A100

Strain S13				*Strain A100*			
Approx No. of viable organisms inoculated	*No. of cows inoculated*	*No. of cows infected*	*No. of cows showing crises*	*Approx. No. of viable organisms inoculated*	*No. of cows inoculated*	*No. of cows infected*	*No. of cows showing crises*
10^9	7	2	1	5×10^8	4	4	1
				5×10^7	2	2	2
10^7	1	0	0	5×10^6	2	1	1
				5×10^5	2	1	1
10^5	1	0	0	5×10^4	4	2	2
				5×10^3	2	0	0
10^3	1	1	1	5×10^2	2	0	0
				5×10	4	0	0
				5	2	0	0

Supplementary Tests for Mastitis

Numerous tests for the presence of mastitis have been summarised by Tompkins, Plastridge and Bryan (1946). As indicated earlier, these tests were variously applied in order to assess their relative value.

Neutrophil counts. The results for the neutrophil counts made before and after inoculation of the infecting strains are given in Table 5 and Fig. 5. From the table it will be seen that 91 per cent of 185 different quarter samples from clinically normal quarters had counts of less than 12,500 per ml. of milk, and that no count exceeded 100,000 per ml.. As recorded in Materials and Methods, inoculations were only made into quarters with negative neutrophil counts. After inoculation of the infecting organism all counts rose sharply and very few were within the normal range at three weeks ; about two thirds were within the normal range at seven weeks. It is of interest that 96 per cent of counts in cases of transient reaction exceeded 12,500 per ml. seven weeks after inoculation although

TABLE 5

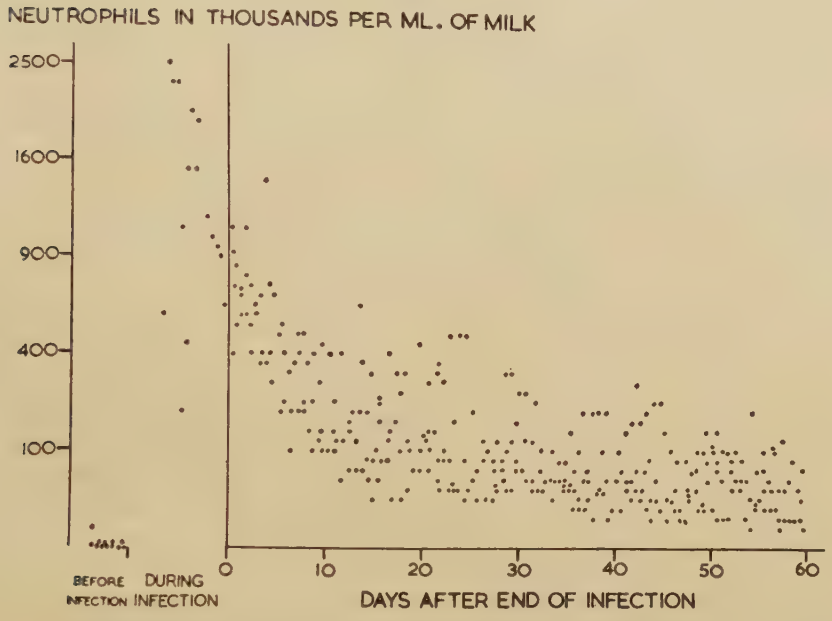
PERCENTAGE DISTRIBUTION FOR TESTS MADE ON MILK SAMPLES BEFORE AND AFTER INOCULATION OF *Str. agalactiae* STRAINS S13 & A100

Type of infection	*No. of examinations*		*Neutrophils in thousands per ml. of milk*										*No. of examinations*		*Whiteside Test Units*					pH						*Rapid Abnormality Indicator*		
	Cows	*Quarter samples*	0	12.5	25	50	100	200	400	800	1600		*Cows*	*Quarter samples*	0	1	2	3	4	< 6.6	6.6 6.7	6.8 6.9	7.0 7.1	7.2 7.3	> 7.3	*Normal (0)*	*Abnormal (5)*	*Marked abnormality (15)*
*Pre-infection samples**																												
From quarters believed to be healthy	35	185	79	12	5	3	1						20	96	91	7	2			15	60	15	8	1	1	95	5	
Counts during 1st week after infection																												
Transient	5	33**					3	12	27	34	24																	
Prolonged	10	81		1	1	6	12	21	24	10	15	10	8	56		7	57	31	5		21	50	25	4		33	65	2
Persistent	13	94		1	0	4	14	25	18	12	18	8	12	84		6	61	31	2		18	43	26	11	2	44	55	1
Counts during 3rd week after infection																												
Transient (no longer infected)	3	36**					42	27	24	7																		
Prolonged	10	80			1	6	20	28	24	16	5		8	56		5	57	38			20	35	34	11		71	29	
Persistent	13	96				1	14	21	32	28	3	1	12	84		1	59	39	1	1	10	35	40	14		75	25	
Counts during 1st four days of 7th week after infection																												
Transient (no longer infected)	3	24**		4	24	30	22	12	8																			
Prolonged (no longer infected)	10	48	21	21	10	17	15	12	2	2			8	32	25	34	41				6	31	41	19	3	44	56	
Persistent	13	56	9	7	7	5	23	22	22	5			12	48	4	4	65	19	8	2		33	21	31	13	28	61	11

* No difference in distribution between quarters carrying saprophytic organisms and those free from saprophytes.

** Sampled twice daily.

Fig. 5



Neutrophil counts (recorded as the square root of the number calculated) given by three cows over a period of two months after the end of a transient infection with S13. The continued abnormality of the counts is well shown.

streptococci had been absent from the milk for at least six weeks ; also that 16 per cent of counts for persistent infection samples were less than 12,500 per ml. at seven weeks although the milk still contained streptococci. The recording of high neutrophil counts over a period of two months in three transient reactions is depicted in Fig. 5, and Fig. 2 shows that abnormal neutrophil counts occurred irregularly in one such case that was under observation for three months.

Whiteside test. From Table 5 it will be seen that 91 per cent of Whiteside tests on milk from clinically healthy udders were negative, and that no reaction of apparently normal milk exceeded two positive units. Inoculations were made only into quarters negative to the Whiteside test. No negative reading was observed in prolonged or persistent infections up to the end of the third week, and only 4 per cent of negative readings occurred in persistent infections at seven weeks. The number of positive reactions fell gradually in prolonged infections until 25 per cent were negative at seven weeks. Our experience has been that this simple test is a most useful indication of abnormality within the udder, and that its readings run closely parallel with the neutrophil count.

pH and chloride measurement. The limited degree of usefulness of

these tests in detecting abnormality within the udder is shown in Table 5. The drawback to both tests, but more especially to the pH measurement, was the wide range of readings obtained on milk from clinically healthy udders.

Agglutination tests. The serum agglutination test showed that no cow had a titre of more than 1:4 before infection and in three cows in which infection was established, about 14 days elapsed before a rise of titre was detected. The titre rose progressively and then became stationary, but in no case was a titre of more than 1:512 to either antigen recorded. Highest titres were obtained with the homologous antigen and there would, therefore, appear to be some type specificity in the agglutination reaction.

In view of the time which has to elapse before a significant rise is detected this test is of no value for a short-term experiment.

Sugar reduction. This test was carried out on all cows inoculated with strain A100 to ascertain whether there was a positive relationship between infection and the power of milk to reduce quantitatively Benedict's solution. In all the cows in which infection was established there was a marked fall in the amount of reducing substance, and the extent of this fall bore a positive relationship to the viable bacterial count. This can be seen in Fig. 4, which also shows that the amount of reducing substance rose as a period of crisis approached. This test would thus appear to be of value in giving an indication of the metabolic activity of *Str. agalactiae* within the udder, and the results suggest that the milk lactose is its source of carbohydrate.

Milk from udders containing saprophytic organisms. When neutrophil counts, Whiteside tests, and estimations of pH and chloride content were being made on the milk of clinically normal udders, a sufficient number of milk samples from quarters harbouring saprophytic organisms was encountered to make possible a comparison between measurements for these milks and for milks free from saprophytes. No significant difference was noted in any of the measurements.

DISCUSSION

One of the objects of this work was to compare the reactions to intramammary inoculation of *Str. agalactiae* in cows and goats. Similarities and differences were noted. The main difference was in species susceptibility for although *Str. agalactiae* infection was readily produced in the goat, quite a high proportion of cows were resistant, a finding consistent with the observations of other workers (Plastridge 1953). The ability of certain cows to resist infection would appear to be a feature of some importance and this aspect of the problem will form the subject of a subsequent paper. Once established, however, the disease process was closely similar in the two species.

The so-called transient reaction noted in some cows inoculated with strain S 13 was not observed in goats ; neither was it observed

in any cows inoculated with strain A100. The host response, as shown by neutrophilic invasion, loss of milk, and febrile response, was more severe following inoculation in this type of reaction and was associated with the rapid elimination of *Str. agalactiae* from the milk. Indeed, the overall picture so closely resembled an immune response that we do not consider this kind of reaction can be considered a valid infection.

Establishment of infection with both strains of *Str. agalactiae* appeared to be related to the number of viable organisms inoculated, and it appears to us likely that strains of *Str. agalactiae* vary in their infectivity, and that there will be variations in the number of viable organisms of different strains required to establish infection. The results obtained with strain A100 indicate that, provided a large enough dose is inoculated, a 100 per cent infection rate can be achieved.

It was noted that a single bacteriological examination of milk might not detect the presence of streptococci, but that if negative samples were obtained on four consecutive days it was likely that the infecting organism had been eliminated from the udder.

The neutrophil response to true infection with *Str. agalactiae* was always considerable and greatly exceeded that caused by physical trauma. The reaction to trauma was transient, whereas that caused by true infection with streptococci sometimes continued after organisms could no longer be recovered from the milk. The neutrophil response to the presence in the udder of non-pathogenic organisms such *Staph. saprophyticus* (Shaw, Stitt and Cowan, 1951) was either minimal or absent.

We attach some importance to the occurrence of crises as they appear to be acute manifestations of an otherwise chronic syndrome. They appear to be a common feature of the disease as they were observed in nine out of 13 infected cows (Table 4). The crisis picture as seen in the cow is essentially similar to that seen in the goat ; in both species the febrile reaction can be of short duration and unless body temperatures are taken every six hours it may be overlooked.

We suggest that the systemic disturbance noted at times of crisis following experimental infection with *Str. agalactiae* and the immune response indicated by a rise of serum agglutinins, make it important to consider mastitis as a disease of the cow as a whole, not merely of the mammary gland. We feel, therefore, that observations made after repeated experimental intrammary inoculation of the same animal at different times must be interpreted with caution.

CONCLUSIONS

In three separate experiments, 35 cows were inoculated once into one quarter of the udder with one or other of two serologically distinct strains of *Str. agalactiae*. The reactions to inoculation were compared with those obtained in goats in similar experiments previously reported.

It was noted that some 50 per cent of cows resisted infection whereas almost all goats had been readily infected, but that once the disease was established in the mammary gland it was apparently the same in both species. The crisis syndrome noted as occurring in about 60 per cent of goats was also seen in about the same percentage of cows successfully infected.

Transient infections associated with inoculation of large numbers of strain S 13 into some cows resembled immune reactions rather than true infections.

Successful infection was apparently related to the number of viable streptococci inoculated, and provided sufficient organisms were inoculated it might be possible to overcome the resistance shown by certain cows.

Absence of streptococci from four consecutive daily milk samples indicated their elimination from the quarter inoculated.

The most useful tests for studying experimental mastitis were considered to be bacterial and neutrophil counts of milk, yield of milk, Whiteside test, and possibly bacterial activity in milk as judged by a sugar reduction test.

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EXPERIMENTAL STREPTOCOCCAL MASTITIS

X. The Importance of a Serum Staphylococcal Hyaluronidase Inhibitor in Infection of the Cow

By

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INTRODUCTION

The experimental introduction of *Streptococcus agalactiae* into the bovine udder has frequently been associated with a low infection rate (Plastridge, 1953). Howell, Pattison, Holman, and Smith (1954) concluded that certain cows possessed some inherent resistance to *Str. agalactiae* and considered that the nature of such resistance should be the subject of further investigation. At the time these studies were being pursued, experiments were also being carried out on the streptococcus itself, with special reference to hyaluronidase production. Examination of the sera of experimentally infected cows had revealed the presence of an inhibitor to the hyaluronidase of *Str. agalactiae*. A further examination showed that certain of these cows also possessed a serum inhibitor to the hyaluronidase of *Staphylococcus aureus*, and the presence of this inhibitor seemed to be related to the type of reaction seen following the introduction of *Str. agalactiae* into the udder. Thus cows which possessed the inhibitor gave a transient reaction whereas cows which lacked the inhibitor gave a crisis-type reaction with the establishment of infection (Howell, *et al*). It was accordingly decided to investigate this relationship more closely and cows were selected as being respectively inhibitor-positive and inhibitor-negative to staphylococcal hyaluronidase and subsequently inoculated with *Str. agalactiae* into the teat sinus.

MATERIALS AND METHODS

The materials and methods for producing infection and for studying the ensuing mastitis were as described by Howell, *et al* (1954). Two strains of *Str. agalactiae* were used viz. strain S13 and strain A100; 21 cows were inoculated with the former and 19 with the latter. The test for the presence of hyaluronidase inhibitor was that of Burnet (1948) carried out in the manner described by Howell (1954).

In assessing whether or not the disease was produced, the establishment of the inoculated strain of *Str. agalactiae* within the udder has been taken as the criterion of infection. For this reason transient reactions were not included as valid infections and crises were especially looked for in view of the importance attached to them as a manifestation of the chronic syndrome (Howell, *et al*).

RESULTS

Observations using Strain S13

The results for 21 cows that received an intramammary inoculation of 10^9 viable organisms are recorded in Table I. These include six cows which gave rise to the original observation.

TABLE I

INFECTION OF COWS WITH 10^9 VIABLE *Str. Agalactiae* STRAIN S13

Cow No.	*Titre of Staph. hyaluronidase inhibitor*	*Whether infection established*	*No. of crises recorded*	*Duration of experiment (days)*
1	1:32	No	—	108
2	1:64	No	—	108
3	1:32	No	—	108
4	1:256	Yes	0	108
5	0	Yes	5	108
6	1:32	No	—	108
7	1:128	No	—	14
8	1:32	No	—	14
9	1:64	No	—	14
10	1:32	No	—	14
11	0	Yes	2	14
12	0	Yes	1	46
13	0	Yes	3	46
14	0	Yes	1	46
15	0	Yes	4	46
16	0	Yes	2	46
17	1:32	Yes	1	46
18	1:64	Yes	0	46
19	1:32	Yes	0	46
20	1:128	Yes	0	46
21	1:64	Yes	0	46

Note: — = no crises as infection not established.

Establishment of infection. In 13 infected cows inhibitor was present in six and absent in seven while in eight non-infected cows, inhibitor was present in every case. This suggests that where the inhibitor is absent there is 100 per cent infection. By the "exact method" $p = 0.015$ for a chance result as favourable to this hypothesis.

Occurrence of crisis in infected cows. In eight cows showing crises the inhibitor was present in one and absent in seven while in five cows which failed to show a crisis the inhibitor was present in every case. These figures strongly suggest an association between the presence of an inhibitor and the absence of a crisis. By the "exact method" $p = 0.0047$ for a chance result as favourable to the hypothesis.

Observations using Strain A100

Table 2 records the results for 19 cows given an intramammary inoculation with strain A100.

TABLE 2

INFECTION OF COWS WITH *Str.. Agalactiae* STRAIN A100

Cow No.	*Titre of Staph. hyaluronidase inhibitor*	*Approx. No. of viable organisms inoculated*	*Whether infection established*	*No. of crises recorded*	*Duration of experiment (days)*
1	0	5×10^8	Yes	1	21
2	0	5×10^8	Yes	0	46
3	0	5×10^8	Yes	0	46
4	0	5×10^8	Yes	0	46
5	0	5×10^8	Yes	0	46
6	0	5×10^8	Yes	0	46
7	1:128	5×10^8	Yes	0	46
8	1:128	5×10^8	Yes	0	46
9	1:32	5×10^8	Yes	0	46
10	1:128	5×10^8	Yes	0	46
11	1:64	5×10^8	Yes	0	46
12	0	5×10^7	Yes	1	10
13	1:64	5×10^7	Yes	1	10
14	0	5×10^6	Yes	1	10
15	1:64	5×10^6	No	—	10
16	0	5×10^5	Yes	1	10
17	1:128	5×10^5	No	—	10
18	0	5×10^4	Yes	1	10
19	1:32	5×10^4	No	—	10

Note : — = no crises as infection not established.

Establishment of infection. In 16 infected cows inhibitor was present in six and absent in ten while in three non-infected cows inhibitor was present in each case. These figures support the result that there is 100 per cent infection in cows lacking the inhibitor but it can be seen from Table 2 that all cows were infected where a large infecting dose was used and the proportion of high infecting doses hides any significance these figures can show by themselves.

Occurrence of crisis in infected cows. In six cows showing crises the inhibitor was present in one and absent in five while in ten cows which failed to show a crisis the inhibitor was present in five and absent in five. This also gives support to the observation made on strain S13 that there is an association between the presence of the inhibitor and the absence of crises. The only cow contradicting this hypothesis is one that received a high infecting dose. Again the numbers are too few for a useful test of significance.

Difference of appearance of milk after infection. Fig 1 shows the macroscopic appearance of the milk from the infected quarters of two cows, one of which possessed the inhibitor and the other which did not. The dose was 5×10^6 viable organisms. The clots present in the milk of the cow in which the inhibitor was absent are clearly seen. A similar difference was noted in the pair of cows receiving 5×10^5 and 5×10^4 viable organisms.

Fig. 1.

Macroscopic appearance of milk three days after inoculation with 5 x 10^6 viable *Str. agalactiae* strain A100. The sample on the left was from cow No. 14 (ear mark 119) and on the right from cow No. 15 (ear mark 508). The gross abnormality of the milk of cow No. 14, which was inhibitor-negative, as compared with cow No. 15, which was inhibitor-positive, can be clearly seen.

Comparison of Results obtained with Strain S13 and Strain A100

It will be seen that the results obtained with the two strains are similar although it would appear that strain A100 was better able to overcome any resistance in the inhibitor positive cows than was strain S13.

As the results are similar for both strains and as the significance of the results in each case depends on a four cell value which in some cells has a theoretical value of less than five, it is convenient to combine the two results.

Establishment of infection. Using either of the two strains a total of 29 cows were successfully infected. Of these inhibitor was present in 12 and absent in 17. In 11 non-infected cows inhibitor was present in every case. From these figures $\chi^2 = 8.9$ and $p = 0.003$ and it would seem reasonable to suppose there is an association between successful infection and the absence of the inhibitor.

Occurrence of crises. Using either of the two strains 14 cows showed a crisis; inhibitor was present in two and absent in 12 of these cows. In 15 cows which failed to show a crisis the inhibitor was present in ten and absent in five. From these figures $\chi^2 = 6.17$ and $p = 0.013$ and it would seem reasonable to assume that there is an association between the presence of the inhibitor and the absence of crises.

DISCUSSION

Howell, *et al.* confirmed the findings of previous workers in demonstrating that certain cows were able to resist experimental infection with *Str. agalactiae*. The observations recorded in this paper though statistically significant raise considerable speculation. One of us (Howell, 1954) has brought forward evidence suggesting that this inhibitor to staphylococcal hyaluronidase is a true antibody and it is unlikely that it could have any influence on the infectivity of *Str. agalactiae*. The findings of McClean (1943) Friou (1949) and Caprio, Rantz and Randall (1952) indicate that a marked antigenic specificity exists between the hyaluronidases of the various cocci. While the nature of resistance to *Str. agalactiae*, which certain cows possess, still remains obscure it appears that it does arise in parallel with the production of an inhibitor to the hyaluronidase of *Staph. aureus*.

CONCLUSIONS

The results of an investigation into the relationship of a serum staphylococcal hyaluronidase inhibitor to the infectivity of *Str. agalactiae* in cows is described.

The experiments involved a total of 40 cows, the serum of 23 of which possessed the inhibitor and that of 17 of which did not. Each cow was inoculated into the teat sinus with *Str agalactiae*.

A significant difference was obtained in the ability of the inhibitor-postive cows to resist infection as compared with the inhibitor-negative cows. There was also a negative association between cows possessing the inhibitor and the occurrence of crises.

It was suggested that it was unlikely that such an inhibitor was directly concerned with the resistance of certain cows to *Str. agalactiae*, but that it occurred in parallel with some other and more important protective factor.

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DISEASES IN WILD BIRDS

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INTRODUCTION

Infectious diseases may be common in wild birds in the British Isles but to date there is no accurate or detailed information available on the subject. It is known, however, that many factors including disease operate to the disadvantage of wild life and cause varying degress of loss. Alternating cycles of dense and sparse wild life populations have been known for centuries but apparently no effort has been made to elucidate the part played by disease as a regulatory factor. This is surprising since as long ago as 1911 Shipley demonstrated that infection with trichostrongyles was a major factor controlling the density of red grouse.

More recently there have been a number of explosive outbreaks of disease in wild birds. Quortrup, Queen and Merovka (1946) described an outbreak of Pasteurellosis in wild duck and Kaschula and Truter (1951) noted an epizootic of fowl cholera in seagulls on Dassen Island. An outbreak of a fungous infection (Aspergillosis) in seagulls has been reported by Herman and Rosen (1947). Avian pox which has been described in many species of birds, (Biester and Schwarte, 1952) was recognised in wild sparrows by McGaughey and Burnet (1945). A viral infection of Manx shearwaters was investigated by Dane (1948) on the island of Skokholm. An epizootic of bronchitis, seen in quail by Olsen (1950), was due to a virus and the condition closely simulated chick bronchitis. McDiarmid (1948) described a form of avian tuberculosis in wood pigeons. He isolated acid-alcohol fast organisms from wood pigeons, rooks and starlings, and described the cultural and pathogenic properties of strains isolated from wild pigeons.

There are also references to isolated cases of disease in wild birds. and many of the more important microbial parasites of man and domestic stock have been isolated e.g. those responsible for Newcastle disease, psittacosis, avian laryngo-tracheitis, avian pox, swine erysipelas, tuberculosis, salmonellosis, pasteurellosis, anthrax and staphylococcosis. In Australia outbreaks of ovine salmonellosis due to drinking water heavily contaminated with bird droppings have been recorded. Magpies, crows and hawks were shown to be infected with *S. typhimurium* and an experimentally infected magpie shed *S. typhimurium* for 21 days after inoculation (Anon, 1952). Neurolymphomatosis has been noted in commercial pheasant flocks in America. (Jungherr, 1939).

METHODS

In order to determine the part played by disease as a cause of death in British birds a survey was commenced in collaboration with

the Public Health Laboratory Service, the Ministry of Agriculture and Fisheries and the British Trust for Ornithology. Each of the regional laboratories undertook to examine dead birds brought in by the general public and to send their results to this laboratory. Routine post-mortem and bacteriological examinations were carried out. In some cases histological examinations were made and where possible helminth and other parasites were identified. In certain cases it was possible to carry out examinations to identify viral agents.

RESULTS

During the period March 1952 to March 1954 a total of 112 birds were examined with the results shown in table 1.

TABLE 1

Species	*No. of Cases*	*Probable cause of death*
Wood Pigeon ..	1	Acid-alcohol fast bacilli. ?*Myco tuberculosis.*
Wood Pigeon ..	3	Pigeon pox
Wood Pigeon ..	1	Unknown
Shelldduck	1	Visceral Lymphomatosis
Shelldduck	1	Unknown
Herring Gull ..	1	Trauma
Herring Gull ..	2	Unknown
Partridge	7	*Pasteurella aviseptica*
Partridge	1	Neural lymphomatosis
Rooks	7	Suspected trinitrocresol poisoning
House Sparrows ..	12	Phosphorus poisoning
House Sparrow ..	1	Unknown
Shearwater.. ..	1	Inanition
Puffin	1	Unknown
Magpie	1	Unknown
Magpie	1	*S. pullorum*
Magpie	1	Unknown
Little Owl	1	Visceral lymphomatosis
Barn Owl	1	Phosphorus poisoning
Stone Curlew ..	1	Trauma
Curlew	1	*S. gallinarum* and a heavy cestode burden
Red Throated Diver	1	Pericarditis
Snipe	1	Trauma
Snipe	1	Trauma
Coot	1	*Pasteurella pseudotuberculosis*
Robin	1	Unknown
Robin	1	Trauma
Song Thrush ..	2	Trauma
Song Thrush ..	1	Unknown
Green Woodpecker	1	Coagulase positive *staphylococci*
Green Woodpecker	1	Trauma
Scaup Duck ..	1	Unknown
Redwing	2	Trauma
Redwing	1	Unknown
Great Tit ..	1	Trauma
Great Tit ..	1	Grorp C *streptococcus*
Great Tits	13	Suspected avian encephalomyelitis
Blue Tits	12	Suspected avian encephalomyelitis
Starling	3	Unknown
Starling	1	*Pasteurella aviseptica*
Starling	1	Group C *streptococcus*
Chaffinch	1	Pulmonary congestion
Chaffinch	1	Trauma
Carried forward	96	

Table 1—continued

Species	*No. of Cases*	*Probable cause of death*
Brought forward	96	
Chaffinch	5	Enterococci
Blackbird ..	1	Trauma
Blackbird ..	1	*S. gallinarum*
Blackbird ..	4	Unknown
Hawfinch	1	Coagulase positive *staphylococci*
Bullfinch	1	Unknown
Brambling	1	Unknown
Skylark	1	Trauma
Meadow Pipit ..	1	Unknown
	112	

Trauma was responsible for 14 cases and bacterial agents for 22. Viral agents were suspected in 31 cases (in only 6 of these was the diagnosis fully confirmed) toxic chemicals in 19 and in 24 cases no diagnosis could be given.

An outbreak of what appeared to be infectious avian encephalomyelitis (epidemic tremor) was observed in a colony of Great Tits and Blue Tits under experimental conditions. Transmission experiments using adult birds were negative but the symptoms, lesions and history of the natural cases were very suggestive. Three clutches of tits were involved and there was a 100 per cent mortality. The incubation period ranged from 7 to 12 days. So far as is known there is no record of this condition in domestic birds in Great Britain.

A strongly haemolytic streptococcus which was classified as a Lancefield's Group C type was isolated from heart blood and bone marrow, of two species (Great Tit and Starling). Coagulase positive *staphylococci* were isolated from heart blood, liver and bone marrow of a Hawfinch and a Green Woodpecker. The only gross lesions in these cases were congestion of the organs and a few petechiae in the heart. Enterococci were thought to have been responsible for deaths in chaffinches kept in an aviary. The chief lesions comprised enteritis and a friable toxic liver. *Pasteurella aviseptica* was the cause of deaths in partridges during a very cold wet spell in March 1952, the lesions produced being pneumonia and a fibrinous pericarditis. *Pasteurella pseudo-tuberculosis* was isolated from multiple caseo-necrotic lesions in the lungs and liver of a coot. Acid-alcohol fast bacilli were demonstrated in granulomatous lesions in the intestine, liver and spleen of a wood pigeon. Morphologically the bacillus appeared to be identical with *Mycobacterium tuberculosis* but no cultural or pathogenicity tests were made. Two common poultry pathogens *S. gallinarum* and *S. pullorum* were isolated from three species, a blackbird, a magpie and a curlew.

Avian lymphomatosis which is a common condition in domestic poultry in Great Britain was noted in three species of birds. Neural

lymphomatosis was seen in a partridge in which the left sciatic nerve and the lumbo-sacral plexus showed gross enlargement and the typical changes were present on histological examination. Visceral lymphomatosis with involvement of the liver and kidneys was seen in a shelduck and a little owl.

CONCLUSIONS

One hundred and twelve dead wild birds have been examined In 24 no cause of death could be found. In some of the remaining 88 a number of human and animal pathogens are believed to have been responsible for disease.

ACKNOWLEDGMENTS

Thanks are due to Professor G. S. Wilson, to the laboratories of the Public Health Service and the Ministry of Agriculture and Fisheries and to the British Trust for Ornithology for assistance in making this survey. These laboratories carried out many of the post-mortem and bacteriological examinations which form the basis of this report.

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STUDIES ON *TRICHOSTRONGYLUS AXEI*

II. The Pathogenesis of *T. axei* in sheep maintained on a high plane of nutrition

By

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INTRODUCTION

In the previous paper in this series (Gibson, 1954) the pathogenic effects of *T. axei* on sheep maintained on a low plane of nutrition were described. Loss of weight and oligocythaemia were the most important symptoms shown, together with a terminal neutrophilia in those animals dying as a result of the infestation. The severity of the symptoms in an individual sheep was apparently related to its inherent resistance. The work has been continued and similar observations have been carried out on sheep kept on a high plane of nutrition; the results of this latter investigation are recorded in the present paper.

MATERIALS AND METHODS

The animals used were six Dorset Horn lambs reared under conditions designed to keep them free from all intestinal parasites except *Strongyloides papillosus* and coccidia and for purposes of the present experiment were regarded as worm-free. Three lambs, (1733, 1766 and 1751) were experimentally infected with *T. axei* whilst the others (1746, 1764 and 1767), were kept as controls.

The experiment was run on similar lines to the previous one except that the diet was different. The origin of the pure strain of *T. axei* has already been described in the previous paper. Observations commenced when the lambs were two months old and, after a preliminary period of three weeks, the three lambs, 1735, 1751 and 1766 each received a dose of 4,000 infective larvae of *T. axei*, repeated daily until a total of 120,000 larvae had been given to each lamb. The diet of the animals consisted of hay and concentrates including bran, flaked maize, crushed oats and linseed cake fed *ad libitum*. During the experiment each was inspected daily for signs of clinical disease and in addition on Mondays, Wednesdays and Fridays, a faecal sample collected from each was examined for the presence of nematode eggs using the Clayton Lane flotation method unless the count was above 100 eggs per gramme when a modified McMaster method was used. On Tuesday a blood sample was examined by the methods described in the previous paper. On Wednesday evenings food and water was withheld until the lambs had been weighed on the following Thursday morning, in order to avoid weight changes resulting from the ingestion of large quantities of food and water.

Lambs which died during the course of the experiment were examined post-mortem on the day of death, whilst two lambs, 1751 (infected) and 1767 (control), which survived to the end of the experiment were slaughtered for post-mortem examination. The other two control lambs were withdrawn from the experiment after 1766 had died, but were not subjected to post-mortem examination.

RESULTS

As the protocols of this experiment are voluminous for purposes of publication they are presented in the form of six diagrams and a table. The complete figures are kept, however, at this laboratory,

TABLE 1.

POST MORTEM EXAMINATIONS OF THE EXPERIMENTAL SHEEP

	No. of Worms Present			
Lamb. No.	*Abomasum*	*Small Intestine*	*Large Intestine*	*Chief lesions*
1746		No post mortem examination		
1764		No post mortem examination		
1767	0	100 *Nematodirus filicollis*	0	Good carcase
1735	22,200 *T. axei*	50 *Strongyloides papillosus*	0	Fair carcase. Severe abomasitis
1766	30,000 *T. axei*	0	0	Poor carcase. Fatty degeneration of liver. Severe abomasitis
1751	1,800 *T. axei*	0	2 Trichuris ovis	Good carcase. Large numbers of "ringworm like" lesions in abomasum.

1746, 1764 and 1767 are controls.

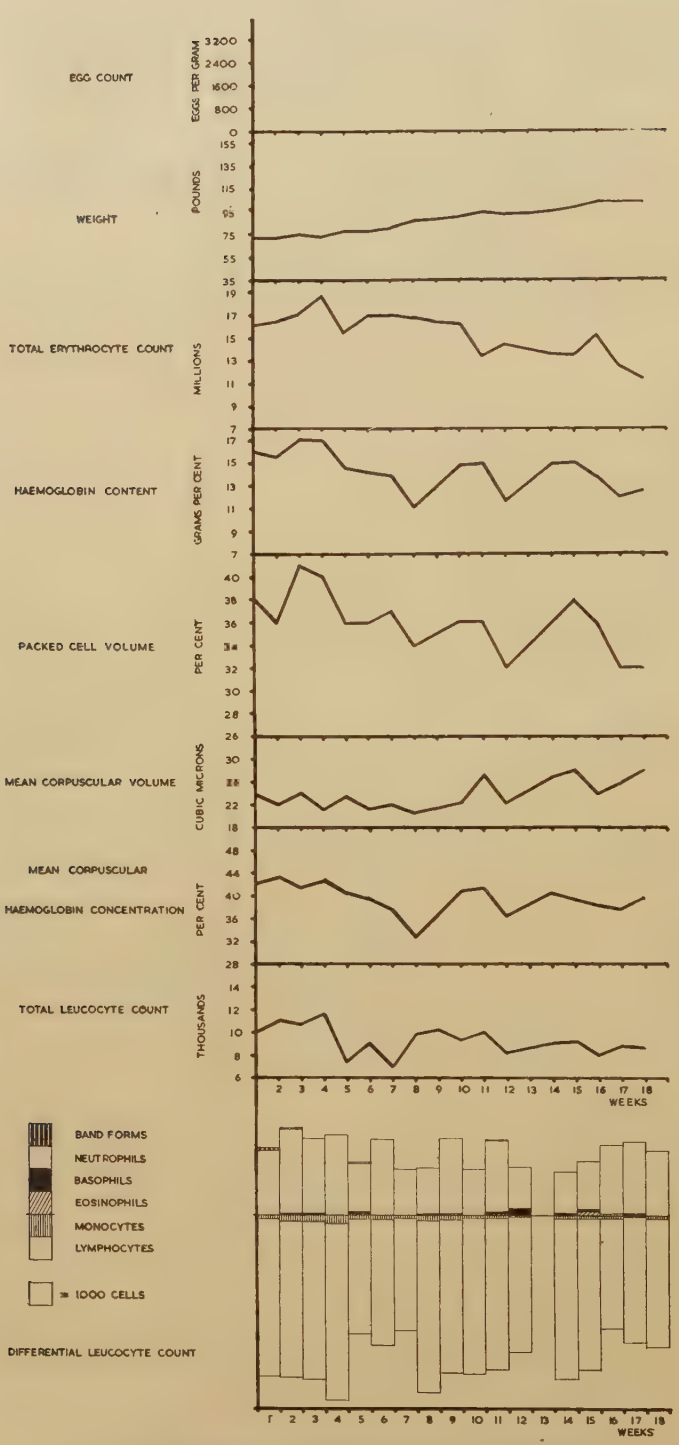
where they may be inspected by anyone who may wish to have fuller details than are given here. The salient points are, however, summarised below :—

The three control lambs (Figs. 1, 2 and 3) gained weight steadily throughout the experimental period. The blood picture showed no change that would not be expected in normal lambs, and the three control lambs may be taken as exhibiting normal reactions under the conditions of the experiment. Only one control lamb, 1767, was slaughtered and examined post-mortem and no lesions were present in the carcase.

One infected lamb, 1735, (fig. 4) died eight weeks after it had received the first dose of infective larvae of *T. axei*. When the worm infestation became patent its rate of weight gain was arrested; it subsequently lost weight, so that at the time of death it was 16 pounds lighter than at the beginning of the experiment. No significant changes in the blood picture were noted. At post-mortem examination the abomasum was found to contain 22,200 *T. axei* the only lesion present being in the abomasum which was acutely inflamed.

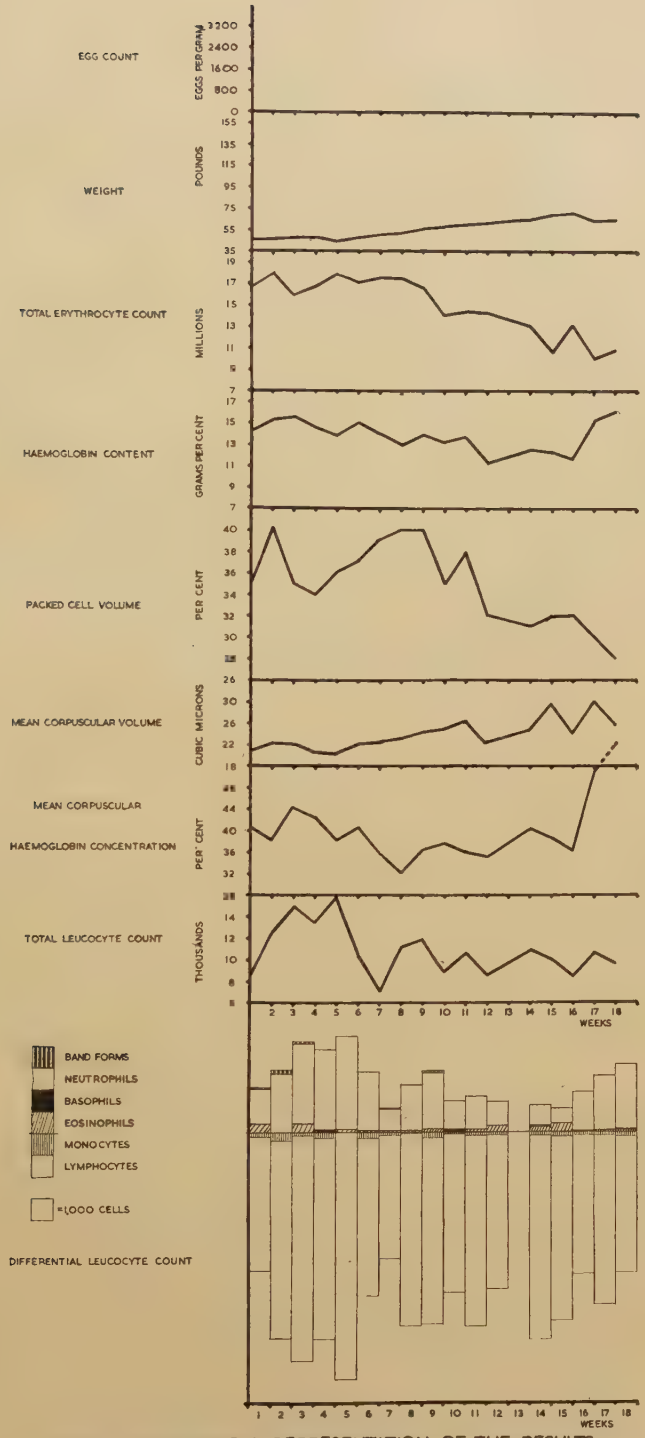
Lamb 1766 (fig. 5) died ten weeks after infection began. It

Fig. 1



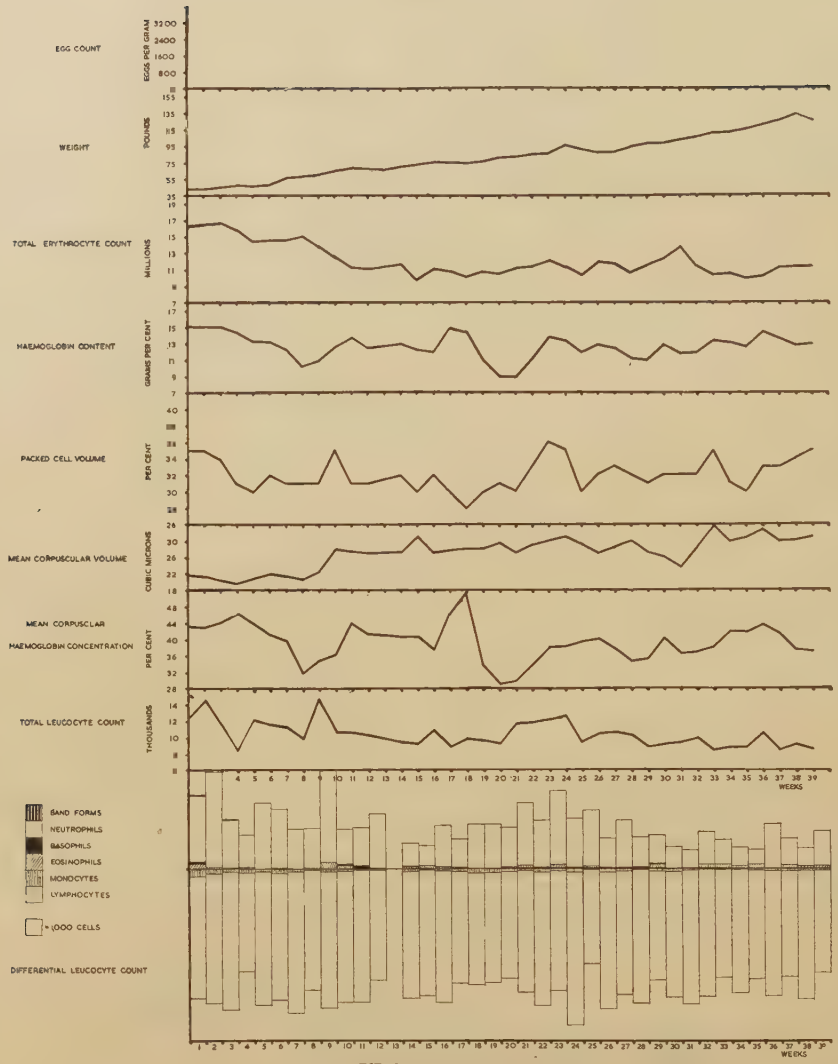
LAMB 1746 GRAPHICAL REPRESENTATION OF THE RESULTS

Fig. 2



LAMB 1764 GRAPHICAL REPRESENTATION OF THE RESULTS

Fig. 3

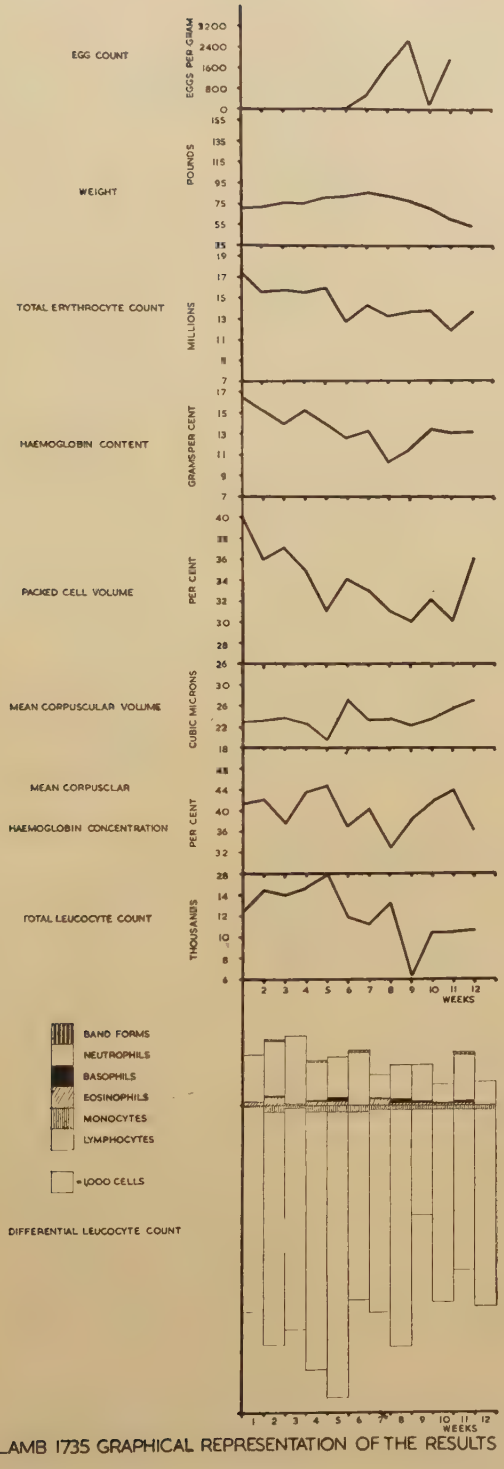


LAMB 1767 GRAPHICAL REPRESENTATION OF THE RESULTS.

increased in weight very slowly for the first nine weeks of the experiment gaining some eight pounds: subsequently it lost weight until at the time of its death it was 11½ pounds lighter than at the beginning of the experiment. No significant changes occurred in the blood picture. Post-mortem examination revealed 30,000 *T. axei* in the abomasum, which was acutely inflamed. Hydropericardium was present and the liver showed fatty degeneration.

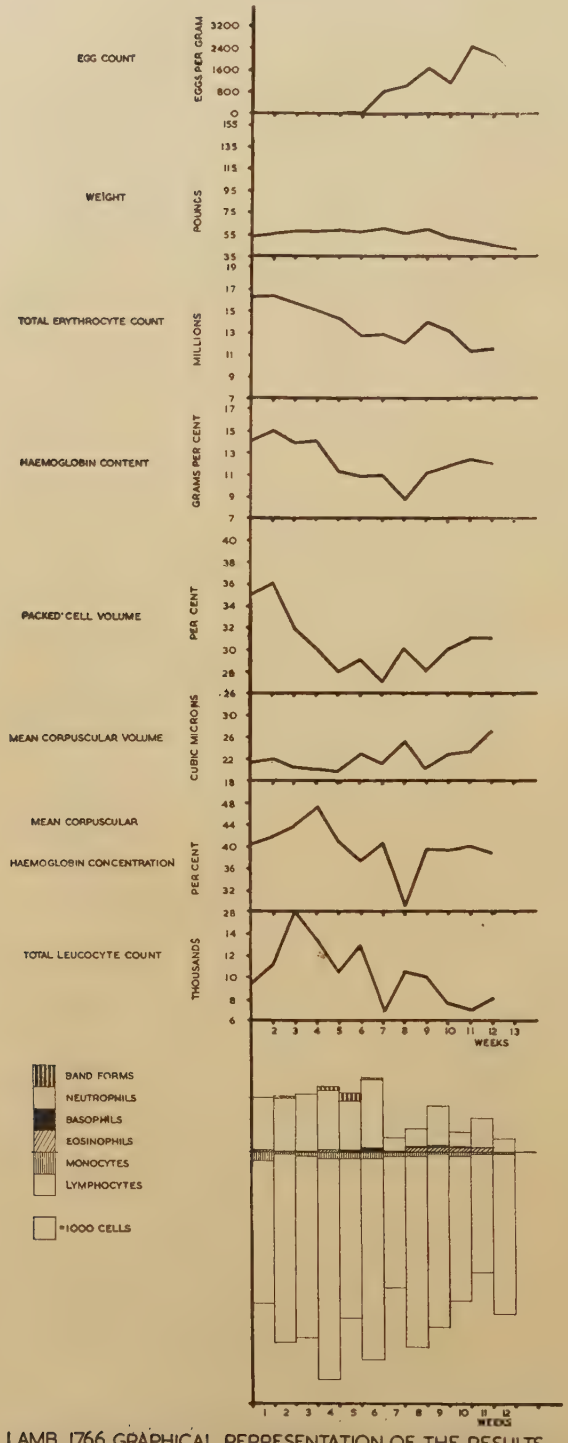
The third infected lamb, 1751, survived till the end of the experimental period. A check in weight gain occurred at the time the

Fig. 4



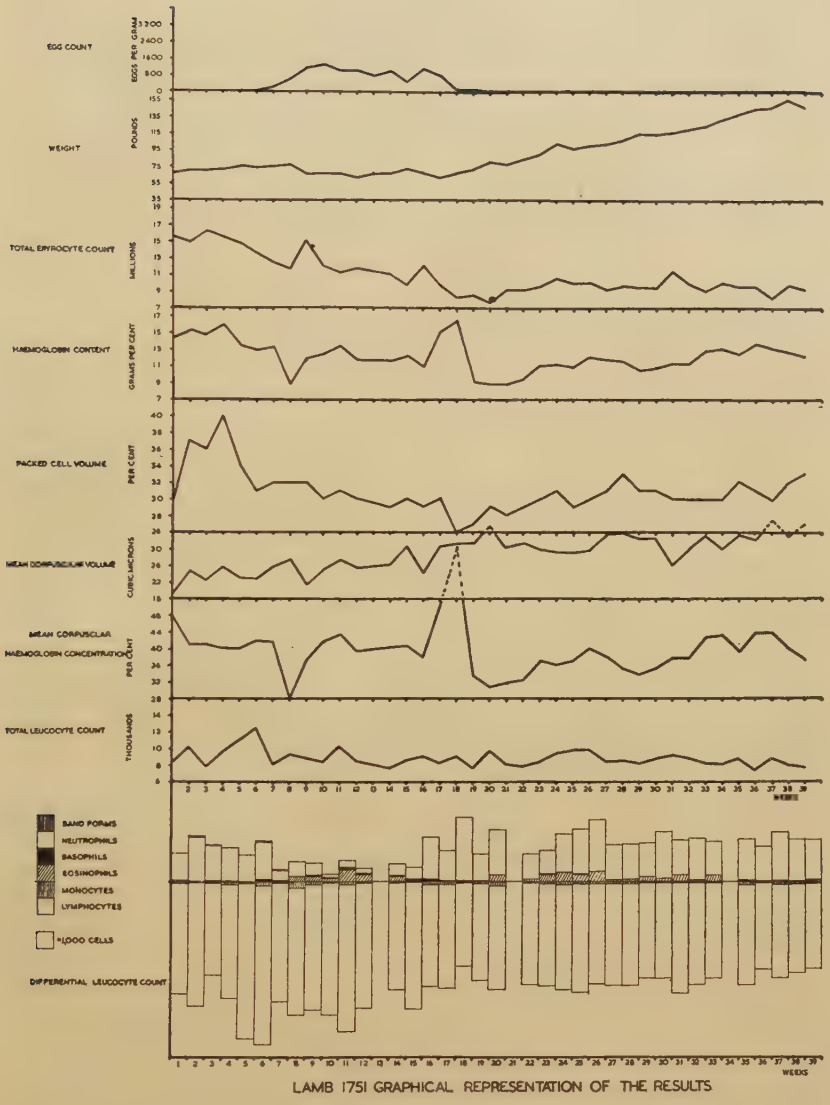
LAMB 1735 GRAPHICAL REPRESENTATION OF THE RESULTS

Fig. 5



LAMB 1766 GRAPHICAL REPRESENTATION OF THE RESULTS

Fig. 6



worm infestation became patent (fig. 6), but later the lamb commenced to gain weight and at the end of the experimental period of 39 weeks it was 50 pounds heavier than at the beginning. Significant changes were not observed in the blood picture and at post-mortem examination the only lesions present were a large number of ring worm-like healed ulcers on the mucous membrane of the abomasum. The worm burden was low, only 1,800 worms being present in the abomasum at post-mortem examination.

DISCUSSION

Many workers have studied the effects of nutrition on gastro-intestinal worm infestations in sheep and their results show that animals on a high plane of nutrition suffer less than others poorly fed. Fraser and Robertson (1933) reported a heavier worm burden in poorly fed lambs than in well fed ones grazing the same pasture and Fraser, Thompson, Robertson and George (1938) showed that when animals were experimentally infected those poorly fed carried more parasites than those receiving a good diet. In lambs dosed with infective trichostronglid larvae Taylor (1943) also found larger numbers of parasites in animals kept on a diet of straw or hay than in those receiving both hay and concentrates.

The results of the present experiment, when compared with the data obtained in the previous experiment in which lambs were kept on a low plane of nutrition, support the view that poorly nourished sheep carry more parasties than do those which are adequately fed. In the previous paper it is recorded that three sheep maintained on a low plane of nutrition died of trichostronglosis, and were carrying worm burdens of 23,650, 55,700 and 57,800 respectively, whilst in the present experiment two sheep succumbed, which, at post-mortem examination, were found respectively to be carrying 22,200 and 30,000 *T. axei*. In the previous experiment two infected sheep survived the experimental period of five months, at the end of which time they had worm burdens of 6,800 and 17,000 respectively. In the present experiment one lamb, which survived to the end of the eight months' period of observation, was found at post-mortem examination to be carrying 1,800 *T. axei*.

These results are therefore in accord with the work of other authors referred to at the beginning of this discussion, but there are one or two points which require comment. It seems, at first sight, anomalous that 30,000 worms should be sufficient to kill lambs receiving a full diet, whereas some 50,000 were required to cause the death of lambs kept on a low plane of nutrition. It is generally accepted that the resistance of lambs to gastro-intestinal nematode infestation increases with age, although age is not the only factor influencing resistance, and this probably provides the explanation. The lambs, kept on a low plane of nutrition, were about nine months old at the beginning of the experiment and received a total of 100,000 infective larvae of *T. axei*. At the beginning of the present experiment, in which the plane of nutrition was high, the lambs were only two months old and they were given 120,000 infective larvae. The younger and more susceptible lambs were exposed to a heavier rate of infestation, and were therefore likely to succumb to a lower worm burden than the older, more resistant, lambs used in the previous experiment. This factor complicates comparison between the two experiments.

The phenomenon of varying degrees of inherent resistance, dis-

cussed in the previous paper, was again observed in the present experiment since of three experimental lambs two died and one survived. The two less resistant lambs which died, had egg counts which increased throughout the duration of infestation and were high at the time of animals' death but in the lamb that survived the egg count first rose to a high level and then fell again and remained low for the last twenty weeks of the experiment. These two types of egg count curve are correlated with low and high resistance respectively; similar observations were made in the previous experiment where the animals were kept on a low plane of nutrition.

In the previous experiment, where the animals were maintained on a low plane of nutrition, changes in the blood picture were a feature of the disease. The animals became anaemic, those which died being more severely affected than others which survived. Several animals also showed neutrophilia a few weeks prior to death. In the present experiment no such changes were observed the blood pictures of both infected and control sheep being similar. An adequate diet was therefore sufficient to prevent anaemia in lambs carrying a heavy worm burden. It is possible, therefore, that the anaemia occurs as a result of the worms abstracting a trace element or vitamin from the intestinal contents of the lambs. Well-fed lambs have sufficient of the particular substance involved but to maintain these conditions both in poorly fed lambs there is insufficient to support both worms and lambs. Although much work would be required to prove this hypothesis, similar phenomena have been described with other helminths; for example Bondsdorff and Gordin (1952) produced evidence to suggest that the macrocytic anaemia caused by infestation with *Dyphyllobothrium latum* is due to the assimilation of large amounts of vitamin B.12 at the expense of the host.

The abomasal lesions caused by *T. axei* varied at different stages of the disease. Animals which died six to eight weeks after infestation showed a very severe abomastitis; the animal which was slaughtered at the end of the experiment showed no abomastitis but a large number of ring worm-like lesions. These consisted of a slightly raised ring of tissue surrounding an eroded area of mucous membrane which had been replaced by scar tissue.

CONCLUSIONS

Artificial infestation with *T. axei* of three two-month old Dorset Horn lambs maintained on a high plane of nutrition produced severe parasitic gastritis from which two of them died. The third lamb survived the infestation but suffered a check in weight gain from the time the infestation became established until resistance was developed.

Haematological examination of the three sheep, together with three similar worm-free controls showed no changes from the normal.

Interesting comparisons are drawn with the previous observations in which infected lambs on a low plane of nutrition developed a marked anaemia, and it is postulated that this anaemia may be caused by worms absorbing large amounts of trace-elements or vitamins from the gut contents of infested sheep.

It was noticed that although severe abomasitis was present in lambs dying from heavy infestations of *T. axei*, the inflammation had subsided in lambs in which the disease was long standing, the striking abomasal lesion in these sheep being ringworm-like ulcers scattered widely over the mucous membrane.

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THE INCIDENCE AND LIFE CYCLE OF *LINGUATULA SERRATA* (FROHLICH 1789) IN GREAT BRITAIN

by

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INTRODUCTION

Linguatula serrata is a member of the family Linguatulidae, the so called tongue worms.

Alcock (1923) has given an account of the attempts to classify the family before Sambon (1922) included them in the phylum Arthropoda being closely associated with the Acarina. Leuckart (1860) first related the adult parasite occurring in the nasal cavity of the dog with the nymph in rabbits, and more recently Von Ratz (1911), Hett (1924), Bugge (1927), Hobmaier and Hobmaier (1940) and Brumpt (1949) have contributed to our knowledge of the parasite. It is generally accepted that the true host of the adult stage is a carnivore while that of the nymph is a herbivore. However, there is evidence to show that the adult may also occur in a herbivore. Thus Sambon lists the following hosts of *L. serrata*—adult stage in man, dog, wolf, fox, horse, ass, sheep, and goat ; nymphs in man, hedgehog, cat, rat, pig, porcupine, guinea-pig, hare, rabbit, horse, ass, ox, sheep, goat, deer, roebuck, antelope, and camel.

In Britain Dick (1840), Hughes (1913), Tutt (1913) and Pillars (1925, 1929, 1932) have recorded the occurrence of adults in the nasal sinuses of the dog but no information concerning the incidence of infestation in this host is available. In other countries, however, the infestation in dogs seems to be more frequent and figures relating to incidence have been recorded by, amongst others, Gaiger (1909), Neumann (1914), Ivanov and Emanuelov (1936), Iwanoff (1937), Gelormini and Roveda (1938) and Marambio (1944).

Pillars (1925) discussing the life cycle of *L. serrata* stated that when the adults have become established in the nasal cavities the female, after fertilisation, lays about 500,000 eggs. These pass to the exterior *via* the nares or in the faeces and come to contain " acariform embryos " which are quite resistant and capable of living for some weeks outside the host. When a suitable host has swallowed the eggs, the shell is digested releasing the larvae which have two pairs of legs and a perforating apparatus. Although it is not definitely known how the larvae reach their nidation site, Brumpt (1949) considered it to be *via* the lymph and blood streams. Boi (1937) and Thornton (1949a) stated that while larvae are found most frequently in mesenteric lymph nodes, they may also be lodged in the liver, lungs, kidneys, spleen, mediastinal, prescapular, iliac, inguinal or lumbar lymph nodes. Most investigators have described the completion of the life cycle by nymphal migration either to the intestine and ultimate escape to the exterior in the faeces, or to the trachea whence the parasite becomes adult in the nasal cavities. Hobmaier and Hobmaier (1940), however, concluded that liberation of nymphs does not occur during the life of the intermediate host.

*Work performed in the Department of Parasitology, School of Tropical Medicine, University of Liverpool.

References to the incidence of the nymphs of *L. serrata* mainly concern animals slaughtered for food, as the parasites are often discovered during routine meat inspection. In Britain they have been reported in cattle by Pillars (1925), Bissett (1926) and Thornton (1949b), the predilection site appearing to be the chain of mesenteric lymph glands. The parasite has received more attention abroad and figures concerning its incidence in cattle and sheep have been recorded by Bogdaschen (1931), Carnevalini (1931), Ivanov and Emanuelov (1936) and Boi (1937).

The present work describes the results of investigations into the occurrence of the adult stage of *L. serrata* amongst dogs and of the nymphs in animals slaughtered at an abattoir. Dogs were experimentally infested with live nymphs and some information was obtained on the development of the adult stage in this host.

Observations on the Incidence of Nymphs of *L. serrata* amongst Animals Slaughtered at an Abattoir in Cheshire

The investigation took the form of two surveys. the first extending from June 1949 to February 1950, and the second from April to September 1950, both being carried out at Bebington abattoir. Because of the difficulty in identifying remains of arthropods and because routine histological examinations were not made, it was decided to base a positive diagnosis on the presence of live nymphs only. In the first survey, samples of mesenteric lymph glands were obtained from adult cattle, calves, sheep and pigs. No attempt was made to obtain any history of the animals from which the glands had been removed or to examine a similar amount of material from each animal. The material was removed to the laboratory and examined as soon as possible after slaughter in the following way :— the glands were separated from the surrounding fat, cut lengthwise, each half squeezed between the fingers and placed in a dish of normal saline at room temperature. Careful watch was kept during the squeezing operation for nymphs which might appear on the cut surface of the gland or on the knife or fingers. Other nymphs not collected during the cutting and squeezing processes escaped into the saline within a few minutes of placing the gland in the fluid.

When a high incidence in adult cattle and a very low incidence or absence of infestation in the other species examined was discovered, it was decided to carry out a more detailed survey of adult cattle only. In the main, animals slaughtered at the abattoir were despatched from Ministry of Food collecting and grading centres. During the investigation it was realized that cattle were being received from three main areas namely Northern Ireland, Eastern Scotland and North-West England with North Wales. However it is important to note that some of the animals did not spend all their lives in a particular area and may have been moved several times. The mesenteric glands of the ox differ somewhat from those of other species for, instead of a chain of small glands, they are mainly composed of elongated portions of up to 12 inches or more in length. In the

TABLE I

THE INCIDENCE OF INFESTATION BY *L. SERRATA* NYMPHS

Three age groups of cattle bought from different areas of Great Britain for slaughter at Bebington

Area	*1—3 years*			*3—6 years*			*Over 6 years*			*Total*	*Total*	
	No. Examined	*Positive*	*%*	*No. Examined*	*Positive*	*%*	*No. Examined*	*Positive*	*%*	*Examined*	*Positive*	*%*
N.W. England and N. Wales	110	24	21.8	228	74	32.5	420	136	32.4	758	324	30.9
East Scotland	42	8	19.0	326	176	54.0	13	9	69.2	381	193	50.7
N. Ireland	52	29	55.8	294	226	76.9	16	8	50.0	362	263	72.4
TOTAL	204	61	29.9	848	476	56.1	449	153	34.0	1501	690	46.0

second survey, each gland together with its enclosing fat was placed in a separate receptacle bearing the reference number of the animal concerned and removed to the laboratory for examination. Details of age, sex and the Ministry of Food centre of origin of the host together with any pathological conditions noticed by the meat inspector were recorded for further reference. After separating the glands from their surrounding fat, a length of about 3 inches was selected from each and examined. If no nymphs were recovered from this first portion of gland after 30 minutes a further 3 inches length was similarly examined.

The Incidence of L. serrata *in different species.* The first survey showed that 30.1 per cent of 681 cattle and 0.42 per cent of 476 sheep were infested. 269 calves (under 1 month old) and 116 pigs were not infested.

Overall and area incidence in bovine infestation. The data in Table 1 show that 46 per cent of 1501 adult cattle slaughtered at Bebington abattoir were infested with the nymphs of *L. serrata.* If the figures from the first survey are included, then 41 per cent of 2182 cattle were shown to harbour live nymphs in the mesenteric lymph glands. This figure is surprisingly high when it is recalled that the presence of the parasite in cattle in Great Britain has been recorded on two previous occasions only. There is little doubt that a more intensive search would show a still higher proportion to be infested. Since the methods of examination adopted on the continent are unknown, comparison of the figures obtained in Cheshire with those of various continental authors is of little value.

The observations here recorded and also those of other authors show that the incidence of *L. serrata* infestation in cattle is fairly high but that there are some area variations. The figures in Table 1 show that the incidence varies from 30.9 per cent in cattle in N.W. England and N. Wales to 72.4 per cent amongst cattle from N. Ireland. In cattle from E. Scotland the incidence was found to be 50.7 per cent but it should be noted that it is a practice in this area to buy cattle from Ireland for fattening and later to send them south for slaughter. These store cattle are slaughtered mainly in the spring and of 381 animals examined from E. Scotland, 52 were known to be of Irish origin. The incidence of infestation in the latter group was found to be 71.2 per cent which approximates closely to the figure given for N. Ireland. If these animals are not included in the total from E. Scotland the incidence in that area falls to 47.4 per cent. The figures for E. Scotland also include 50 local bred cattle from Maud, none of which showed infestation. This centre is a little further north than the others in the area, and is the only one to show completely negative results.

Estimations of the density of infestation were not attempted but there appeared to be only little variation between the different age groups. In areas of higher incidence of infestation there was some

indication that larger numbers of nymphs were recovered from similar portions of gland.

Age and sex incidence in bovine infestation. The animals from which material was examined were placed in the following age groups, 1 to 3 years, 3 to 6 years and over 6 years. The data in Table 1 show that of the total number of animals examined, the highest incidence was in the middle age group. The lower incidence amongst older animals was probably not a true representation of the position, as only small numbers of Scottish and Irish cattle of this age group were examined. It should be noted, too, that in the case of animals from N. W. England there was little difference between the incidence of infestation in the middle and older age groups. The lower incidence in younger animals was not unexpected and probably reflects shorter exposure to infestation.

THE FINAL HOST OF *L. Serrata*

In view of the high incidence of infestation disclosed by the surveys in cattle, it was decided to investigate the incidence of the adult stage in dogs. Diagnosis of the conditions in dogs is unsatisfactory unless the animal is kept under observation for some time and thus it was decided to examine dogs destroyed at the Liverpool Dogs' Home. The nasal chambers of 206 dogs were searched for *L. serrata* and in no instance was the parasite present.

If the theory advanced by Hobmaier and Hobmaier (1940) that the nymphs do not leave the intermediate host during its life, is correct, then, for a dog to become infested, it must have access to slaughter-house offal or to the carcase of an infested herbivore. The negative result mentioned above is not, therefore, surprising, as it is doubtful if the city-reared dogs used in the survey would have the opportunity of becoming infested by either method. There is the possibility of small herbivores such as rabbits and hares, acting as the intermediate host of *L. serrata,* and in this case it is likely that the adult stage would occur in sporting and country dogs or in wild carnivores such as the fox. That the fox can play a part has been shown by Griffiths and Sinclair (1953) when 3 out of 150 examined in N. Ireland were found to harbour the parasite.

THE EXPERIMENTAL ADMINISTRATION OF *L. Serrata* NYMPHS IN DOGS

As two hosts are required for the completion of the life cycle of *L. serrata,* then the nymphs in the glands of the intermediate host must reach the nasal cavities of the definitive host. Different authors have suggested various routes by which the nymphs may gain access to the nasal chambers. Thus, Leuckart (1860), Koch (1906) and Heymons (1935) thought the nymphs climbed up the oesophagus following ingestion. Colin (quoted by Pillars 1925) and Southwell (1924) believed the nymphs were inhaled, while Gerlach (also quoted by Pillars 1925) thought the nymphs reached the nasal cavities follow-

ing penetration of the stomach wall. Hobmaier and Hobmaier (1940) in their experiments failed to infest dogs by inhalation and they also failed to prove the theory of migration up the oesophagus. These authors concluded that nasal infestation followed the ingestion of infested food, either directly through free nymphs or those liberated in the mouth, or indirectly through nymphs regurgitated from the stomach in the act of vomiting.

Most of the dogs used in this investigation were obtained from local dealers and were from 6 to 12 weeks old. However one or two animals were about 12 months old and some were bred in the kennels from stock bitches at the Liverpool School of Tropical Medicine. The method of isolating the nymphs has already been described. They were kept in normal saline in Petri dishes at room temperature until required and only active nymphs were used. The nymphs were used as soon as possible after isolation from the glands, mainly within 24 hours. The feeding experiments extended at irregular intervals over a period of approximately 12 months.

TABLE 2

THE REACTION OF CONSCIOUS FED AND FASTED DOGS
After the administration of *L. serrata* nymphs in meat

	Dog No.	*No. of nymphs fed*	*Interval between feeding & reaction*	*Reaction*	*Examination of vomitus*
Fed	1	12	30 minutes	vomited	negative
	2	10	45 ,,	,,	positive (2)
	3	21	15 ,,	,,	negative
	4	7	30 ,,	,,	,,
	5	12	no reaction	—	—
	6	18	20 minutes	vomited	negative
	7	50	20 ,,	,,	,,
	8	20	30 ,,	,,	,,
	9	15	5 ,,	,,	,,
	10	15	15 ,,	,,	,,
Fasted	11	20	20 ,,	coughed	—
	12	20	15 ,,	,,	—
	13	5	15 ,,	,,	—
	14	25	15 ,,	,,	—
	15	25	45 ,,	,,	—

In the first series of experiments, each dog was fed pieces of lightly cooked meat containing known numbers of nymphs. The " baited " meat was usually given after the dogs had eaten one or two pieces of meat. This method was adopted in an effort to persuade the dogs to swallow quickly the " baited " meat and to reduce the possibility of parasites being released in the mouth. For reasons to be indicated later, both fed dogs and dogs which had been fasted overnight were used. After the experimental feed the dogs were observed for about an hour or until any reaction to the infested meat had ceased. All vomited material was collected, washed and sieved, and examined for parasites.

The results of these experiments are shown in Table 2. After the administration of live nymphs to fed dogs in all cases except one vomiting occurred. This vomiting reaction took place at any time between 5 and 45 minutes after administration. It will be seen from the Table that the number of nymphs fed had no apparent influence on the period that elapsed between feeding and vomiting, and that in one case only were the parasites found in the vomitus.

In an attempt to eliminate vomiting, it was decided to observe the reactions of fasted dogs after feeding with nymphs. Table 2 shows that the administration of nymphs to fasted dogs caused a reaction in all cases. The period that elapsed between the feeding and the onset of reaction was, in general, shorter than in fed dogs. The reactions observed were symptoms of nausea, the animal licking the lips, perhaps yawning, and appearing uneasy. This was followed by a few recurrent attempts to vomit, the animal opening its mouth and coughing as though an irritation in the throat were present. This coughing recurred at intervals for varying periods, the longest time noted being $2\frac{1}{2}$ hours. As will be seen later, the duration of the reactions appeared to be dependent upon the location of the parasites. The initial nausea and vomiting attempts were doubtless due to the presence of the nymphs in the stomach but, at autopsy, parasites were often found in the larynx and their presence there would probably account for the extended period of coughing. It is not clear whether the coughing started when the nymphs reached the pharynx or whether it was associated with gastric irritation. In either case the period of nausea was usually short before the onset of coughing and if the latter began only when the nymphs reached the laryngeal region, then they must have arrived there relatively quickly. For the sake of convenience the word " coughed " has been used in Table 2, and later in Table 3, to describe this reaction.

In order to ascertain whether a toxic product of the nymphs was responsible for the reactions observed, six dogs were each dosed with a mixture of 25 nymphs ground up in 2 ml. of normal saline. In no case was there any response and all the dogs so dosed later reacted, as described above, when live nymphs were fed to them.

In the second series of experiments a different method of administration was used. This involved the use of a rubber stomach-tube of $\frac{1}{4}$-inch internal diameter and a No. 4 gelatin capsule. The required number of parasites was transferred with a fine brush to the bottom portion of a capsule, care being taken that as little fluid as possible was included with them, and the top of the capsule replaced. The charged capsule was then pushed through the stomach tube by another piece of rubber tubing sliding within it. It was found to be essential to administer the capsule as soon as it had been charged as even the small amounts of saline transferred with the nymphs during the loading process caused the gelatin to soften and swell with the result that the capsule would not pass down the tube. Experiments

in vitro using synthetic gastric juice at 37°C showed that the contained nymphs would all be released from the capsule in about seven minutes. This method of administering the nymphs was found to be the most satisfactory one since it was possible to place a given number of nymphs in the stomach and to trace their migrations by autopsy at predetermined times after administration.

All the dogs used in this series were fasted overnight before the nymphs were administered. Of a total of 26 dogs, 14 were conscious throughout, and the remaining 12 were anaesthetised before the capsules were given. Any reactions of the dogs were noted and the results are shown in Table 3. In the 12 anaesthetised dogs nembutal was administered intravenously or intraperitoneally in order to maintain a medium depth of anaesthesia up to the time of killing. Thus it was possible to compare the extent of migration of the nymphs in conscious and anaesthetised dogs, and also to ascertain

TABLE 3

THE REACTIONS AND THE RECOVERY OF PARASITES.

Autopsy of fasted, conscious and anaesthetised dogs following the administration of nymphs of *L. serrata* in a gelatin capsule through a stomach-tube.

	Dog No.	No. nymphs admind	Reaction	Time between admin. & reaction	Time between admin. & P.M.	D	S	O	L	TI	M	PM	NC	T	%
Conscious	(1)	20	coughed	10 mins.	½ hr.	—	14	4	—	—	—	—	—	18	90
	(2)	20	,,	20 mins.	1 hr.	—	16	1	—	—	—	—	—	17	85
	(3)	20	nil	—	1 hr.	—	13	1	—	—	—	2	—	16	80
	(4)	20	coughed	30 mins.	1 hr.	—	14	1	—	2	1	—	—	18	90
	(5)	20	coughed	15 mins.	1½ hrs.	1	10	2	—	4	—	3	—	20	100
	(6)	20	coughed	15 mins.	1 hr.	—	—	2	6	5	—	3	—	16	80
	(7)	20	coughed	45 mins.	1½ hrs.	—	7	5	—	1	2	1	—	16	80
	(8)	20	coughed	15 mins.	2 hrs.	—	13	3	—	1	1	—	—	18	90
	(9)	20	coughed	30 mins.	2½ hrs.	—	11	1	—	—	2	1	1	16	80
	(10)	20	coughed	25 mins.	3½ hrs.	1	3	1	(10 in small intestine)					15	75
	(11)	20	coughed	20 mins.	4 hrs.	—	3	1	2	2	3	2	—	13	65
	(12)	20	coughed	10 mins.	4 hrs.	—	8	1	1	4	—	2	—	16	80
	(13)	17	coughed	40 mins.	4½ hrs.	—	3	—	4	3	—	—	3	13	77
	(14)	20	coughed	15 mins.	5 hrs.	—	4	—	2	3	3	3	—	15	75
Anaesthetised	(15)	20	nil	—	¾ hr.	—	13	7	—	—	—	—	—	20	100
	(16)	20	nil	—	1½ hrs.	—	6	12	1	—	—	—	—	19	95
	(17)	20	nil	—	2 hrs.	—	17	2	—	—	—	—	—	19	95
	(18)	20	nil	—	2 hrs.	—	16	2	—	—	—	—	—	18	90
	(19)	20	nil	—	2 hrs.	—	20	—	—	—	—	—	—	20	100
	(20)	20	nil	—	3 hrs.	—	8	6	—	—	—	2	—	16	80
	(21)	20	nil	—	3 hrs.	—	12	7	—	—	—	—	—	19	95
	(22)	20	nil	—	4 hrs.	—	13	2	1	—	—	1	—	17	85
	(23)	20	nil	—	4½ hrs.	—	4	6	1	2	—	1	2	16	80
	(24)	20	nil	—	4¾ hrs.	—	16	1	—	1	—	—	—	18	90
	(25)	20	nil	—	5½ hrs.	1	4	10	—	1	2	—	—	18	90
	(26)	20	nil	—	6¼ hrs.	—	3	7	1	1	1	2	—	15	75

Key D — Duodenum
S — Stomach
O — Oesophagus
L — Larynx
TI — Tonsil
M — Mouth
PM — Posterior meatus
NC — Nasal Cavity
T — Total recovered

whether or not the migration was affected by any reaction on the part of the host. In the conscious dogs a similar reaction was observed to that previously noted in the first series of experiments. In the anaesthetised dogs there was no reaction.

Table 3 shows the positions of the nymphs found at autopsy of conscious and anaesthetised dogs, which were killed at varying times after administration of the nymphs. The data show that a proportion of the nymphs liberated in the stomach of a dog arrive eventually in the nasal cavities. From the varying positions in which the nymphs were found at autopsy, the route of migration appears to be up the oesophagus where the nymphs are found at different levels, and then into the pharynx where they lodged in any suitable crevice or fold. They also occurred in the tonsillar region both in the crypts and under the fold of mucosa adjacent to the tonsils and in the larynx either within its cavity or in the ventricles. The indications are that they pass along the roof of the pharynx to the posterior meatus in order to reach the nasal cavities where they eventually attain maturity.

TABLE 4

THE NUMBER AND GREATEST LENGTH OF *L. Serrata* ADULTS

Autopsies performed at different intervals after the administration of nymphs to conscious dogs

Dog No.	*No. of nymphs admin.*	*P. M. time in weeks*	*No. of adults*	*Length of largest adult*
1	12	11	5	14 mm.
2	16	15	5	20 mm.
3	8	16	2	15 mm.
4	20	20	10	24 mm.
5	21	42	2 (males)	10 mm.
6	21	56	2 (males)	10 mm.
7	20	56	9	34 mm.

In considering the mechanism of the migration, there seems to be little doubt that the parasite is able to complete its migration independently of vomiting or other noticeable reaction on the part of the host. However in the conscious dog in which there is a reaction, a greater proportion of nymphs reach the head and a shorter time is required for the migration. Table 3 shows that a nymph was recovered from the nasal cavities of a " conscious " dog $2\frac{1}{4}$ hours after administration of the capsule. In an anaesthetised animal nymphs were found in the nasal cavities at autopsy carried out $4\frac{1}{2}$ hours after administration. Further investigation into the mechanism of migration is needed for, although movements of the alimentary

tract may play some part in retarding or assisting the nymphs or in indicating the route of migration, it is possible that some other factor is involved. There is, however, no doubt that the nymphs are capable of quite rapid movement using the four large anterior hooks.

Table 4 shows the numbers and greatest lengths of the adult form of *L. serrata* recovered at autopsy of experimentally infested dogs killed at varying periods of time. The rate of growth of the parasites within each dog was not uniform, one or two specimens being larger than the rest. The adults seemed to occur almost anywhere within the nasal cavities appearing to favour sites away from the mid line. They were often found concealed within folds of the turbinate bones adjacent to the inner surface of the superior maxillae high up towards the frontal sinus. After longitudinal section of the head it often happened that no parasites were at once seen, and the contents of the nasal chambers had to be carefully searched before they were visible.

Hobmaier and Hobmaier (1940) stated that the parasites in the dog reached maturity about six months after infestation and that the eggs began to appear in the nasal secretions at about this time. The method of demonstrating eggs adopted in the present investigation was to roll a small piece of cotton wool around a pair of forceps and to push the swab as far as possible up each nostril. A fresh swab was used for each nostril and a wet smear prepared for microscopical examination. Nasal swabs were examined twice a week from the tenth week of infestation up to the time of autopsy. When eggs were observed, daily swabbing was introduced.

In the series of dogs described in Table 4, eggs were only recovered from two by this method. In the case of dog No. 7, eggs first appeared during the 32nd week after infestation, and for the next three weeks one or two eggs were observed regularly on swabs from the right nostril only. At this point the number of eggs suddenly increased and on one day as many as 70 were found on one swab. A few days later single eggs began to appear from the left nostril and continued to do so for about a week. The discharge of eggs then ceased and no further eggs were observed during the following four weeks by which time 40 weeks had elapsed since the nymphs were administered. Between the 40th and 45th week eggs occurred irregularly, but after this, no eggs were recovered from this dog which was autopsied in the 56th week. The largest female parasite found in this dog was 34 mm. long. In dog No. 6 an egg was first seen during the 35th week after infestation and for a period of five weeks, eggs were found irregularly from the left nostril only. Eggs were still being discharged irregularly when the dog was killed in the 56th week. Post-mortem examination revealed two male adults only. The female parasites had apparently left the dog prior to autopsy. An examination of the last column in Table 4 shows that there is a

more or less steady increase in length of the parasites relative to the duration of the infestation.

The eggs have thick yellow shells and are oval in shape. They measure about 90μ x 70μ and when laid are enclosed in a thin, transparent loose envelope which may be lost during the swabbing. Within the egg the larvae were observed in various stages of development.

No symptoms of nasal irritation were apparent in any of the dogs used in this experiment but it is possible that the parasites were not sufficiently large or numerous to produce irritation or obstruction.

DISCUSSION

The differences in the incidence of *L. serrata* nymphs amongst different host species may be due to one or more reasons. There may be varying susceptibility although it is apparent from the figures given by continental authors that in some areas sheep are as susceptible as cattle. No figures are available on the incidence of infestation in pigs but in this species different methods of husbandry may be a factor. Age at slaughter and methods of husbandry are probably the main factors responsible for the distribution of the parasite in the different host species examined. It has been shown that the incidence of *L. serrata* nymphs in cattle in certain areas is as high as 72 per cent, and it is probable that had more gland material been examined from each animal, a still higher rate of infestation would have been discovered. In this work it has been assumed that infestation is transmitted *via* the pasture only, but the possibility of other modes of transmission including pre-natal infestation cannot be ruled out.

No figures are available on the occurrence of the adult stage amongst dogs in this country but in a small survey carried out amongst urban dogs in Liverpool not a single case of infestation was found. Whilst it is possible for one or two adult parasites to produce sufficient eggs to contaminate a considerable area of ground it is probable that in Northern Ireland there is a widespread infestation in the final host. The number of carnivores with access to bovine mesenteric glands must be very small and this seems to suggest that the bovine infestation is merely accidental. Pullar (1936) in Australia advanced the hypothesis that *L. serrata* is propagated in that country principally by the fox—rabbit—fox sequence and a similar cycle may go on in Great Britain.

The eggs appear to be discharged irregularly in the nasal secretions and are probably concentrated, for some time at least, over a relatively small area of ground owing to the mucoid nature of the secretions. It seems unlikely that the eggs hatch before ingestion by the intermediate host and it may be that large numbers of eggs are occasionally ingested at a time. The precise route by which the larvae reach the lymph glands is still obscure. No further light has

been thrown on this by the writer as nymphs were not recovered from any site other than lymph glands. When the large numbers of nymphs which may occur in some portions of gland, as many as 60 in 3 inches of mesenteric gland, are considered, it seems strange that any large scale migration through the liver and lungs, if it occurs, has not been noted at autopsy.

It is apparent that there are hazards to the completion of the life cycle of *L. serrata* and it must be on few occasions that the nymphs in the lymph glands of cattle eventually reach the nasal cavity or even the stomach of a dog. Rarely, the cycle can apparently be completed in one host, a herbivore, in which the nymphs reach the nasal cavities either during migration in the larval stage or by direct penetration in the nymphal stage. Whether the nymphs reach the definitive host from small herbivores such as rabbits or hares, or by other means, it appears that both sexes of parasite must be present for the production of fertile eggs.

CONCLUSIONS

A survey of the incidence of live nymphs in mesenteric lymph glands of animals slaughtered at Bebington (Cheshire) abattoir showed that 30.1 per cent of 681 cattle and 0.42 per cent of 476 sheep were infested. 269 calves (under 1 month) and 116 pigs were not infested. In a survey which included adult cattle only, an area variation was shown in the incidence of infestation, viz : 72.4 per cent in 362 animals from N. Ireland, 50.7 per cent in 381 from E. Scotland and 30.9 per cent in 758 from N. W. England and N. Wales.

No *L. serrata* adults were found in the heads of 206 dogs examined from the Liverpool area.

Live nymphs were administered to experimental dogs and observations were made on the migration of the parasite from the stomach to the nasal cavities. It was found that the migration of the nymphs was assisted by the reaction of the host.

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A COMPARISON OF THE INTRADERMAL AND SUBCUTANEOUS ROUTES IN PRODUCING IMMUNITY TO BRUCELLOSIS IN CATTLE.

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INTRODUCTION

During the last ten years, several experiments to investigate immunity against brucellosis have been conducted at this Field Station (Edwards, de Ropp and McLeod, 1945 ; Edwards, McDiarmid, de Ropp and McLeod, 1946 ; McDiarmid, 1949, 1950). Using the standard technique adopted in these experiments, McDiarmid (1950) showed that 0.2 ml. of S. 19 vaccine, given by the intradermal route, created an immunity equal to that produced by a large dose (5.0 ml.) injected subcutaneously. When this preliminary experiment was commenced sufficient cattle were not available for the inclusion of a group vaccinated with the same amount namely 0.2 ml. S. 19 by both routes, although it was fully realised that such a comparison with the same dosage should have been made. Later, further experimental animals became available and this paper describes the results of comparing the immunity produced by this small dose injected by both routes with the more commonly employed dose of 5.0 ml. subcutaneously.

MATERIALS AND METHODS

Animals. These consisted of forty Ayrshire heifers reared in a non-vaccinated brucellosis-free herd and all gave negative results to the agglutination test on two occasions prior to vaccination. The heifers were about two months in calf at the time of vaccination and in this respect differed from animals, used in previous experiments, which had been vaccinated prior to service ; four groups were formed, ten animals in each group, and these were housed in separate isolation units of the type described in previous papers (Edwards, *et al.*, 1946).

Vaccination. Strain 19 *Br. abortus* vaccine (Ministry of Agriculture standard issue) was inoculated as follows :—

Group **1**. Ten heifers received a dose of 0.2 ml. injected subcutaneously into the thoracic region just posterior to the olecranon process.

Group **2**. Ten heifers received a dose of 0.2 ml. injected intradermally at the same inoculation site as in Group 1.

Group **3**. Ten heifers received a dose of 5.0 ml. inoculated subcutaneously in the same position as the previous two groups. This group was included in order to determine the protective power of the vaccine, in a dose which already had proved effective in past experiments at this Field Station.

Group **4**. Ten non-vaccinated heifers which formed a control on the infectivity of the test dose.

Exposure to infection. At the time of infection and three months after vaccination, the heifers were approximately five months in calf. The procedure adopted was the same as in previous experiments i.e. 0.1 ml. of a suspension estimated to contain approximately 150 million viable *Br. abortus,* strain 544 was instilled into the conjunctival sac of each animal. The viability and virulence of this infecting suspension was checked by plate counts and by the inoculation of guinea pigs. The results of the tests in guinea pigs are shown in Table 1, and the actual viable count showed the infective dose to contain 134 million organisms.

TABLE 1

VIRULENCE TEST OF INFECTIVE DOSE

10 Guinea Pigs inoculated intramuscularly with 3 x 10^6 strain 544.

16 days			*9 weeks*		
Agglutination titre	*% Spleen wt./ Body wt.*	*Approximate number of* Br. abortus *in spleen*	*Agglutination titre*	*% Spleen wt./ Body wt.*	*Approximate number of* Br. abortus *in spleen*
2560	0.16	265,000	81920	2.8	13,000
1280	0.19	175,000	20480	0.45	2,000
2560	0.19	198,000	5120	0.20	3,000
1280	0.14	180,000	1280	0.16	4,000
1280	0.19	142,000	Died	—	—

Agglutination tests. Blood samples from the heifers were examined frequently for the presence of agglutinins. The technique has similar to that used on previous occasions (Edwards *et al.,* 1946).

Examination of materials collected at parturition. The technique was been described in our previous papers. Cotyledons, foetal stomach contents when available, colostrum and milk samples were examined culturally and biologically ; in addition, the blood serum agglutination titre of each animal was recorded at parturition. Milk samples from the individual animals were tested for the presence of *Br. abortus* each week for a period of ten weeks after calving.

RESULTS

General Response to Vaccination

Small dose (0.2 ml.) subcutaneously. In this group no marked systemic reactions were noted. The local oedematous areas varied from 1 in. to 2 in. in diameter and were small in comparison with the typical picture following the injection of 5.0 ml., while one of the heifers showed no abnormality at the inoculation site.

Small dose (0.2 ml.) intradermally. The reactions associated with this type of injection have already been described in detail, (McDiarmid, 1950) and the present results obtained in this group confirmed these

TABLE

COMPARISON OF IMMUNITY PRODUCED BY VARIOUS DOSES

Group	Number of heifer	Service to vaccination (days)	Agglutination titres of serum: Weeks prior to vaccination		Weeks after vaccination				Infective dose applied	Weeks after infection						
										4	8	12	16	20	24	28
			1	0	1	2	3	4	8	12	16	20	24	28	32	36
1 0.2ml. S.C.	49502	99	N	N	4	8	8	7	4	8	8	10	11	10		
	49464	121	N	N	3	8	6	6	3	4	5	8	7	7		
	54810	106	N	N	5	9	9	9	3	5	5	5	5	4		
	95350	106	N	N	2	3	3	3	1	1	1	N	1	1	1	
	1418	95	N	N	5	8	8	7	4	4	4	4	4	4	5	5
	54803	81	1	1	3	12	12	12	9	7	8	9	9	8		
	26008	80	1	1	4	8	8	7	5	7	6	6	6	5	9	
	70351	79	N	N	5	9	10	8	3	3	3	2	2	2	2	2
	54828	75	1	1	5	12	14	13	8	6	6	6	6	7	7	7
	545	91	1	1	4	6	3	5	2	1	N	1	1	1	1	
2 0.2ml. I.D.	54796	101	N	1	6	13	11	9	7	7	9	9	10	9	7	
	482	101	N	N	6	10	9	10	7	7	7	8	6	6	7	7
	520	95	N	N	6	9	9	8	4	3	3	3	3	3	4	3
	25974	100	N	N	7	10	10	9	6	6	6	7	6	6		
	541	106	N	N	7	11	11	9	6	6	7	8	6	6		
	25992	102	N	N	5	10	10	8	6	6	8	10	10	8	8	
	70345	102	N	N	6	12	12	11	10	8	7	7	7	7	7	
	49493	73	N	1	5	14	12	13	8	8	8	8	7	7	8	8
	26013	74	N	1	6	10	10	10	6	6	6	6	7	7	7	7
	81656	83	N	N	6	8	6	6	3	2	2	2	2	Dead		
3 5.0ml. S.C.	54788	99	N	1	7	13	11	13	10	7	7	8	8	8	6	
	26016	94	N	N	7	11	12	11	9	6	6	7	8	10	11	
	70346	101	N	N	6	13	11	11	9	7	8	7	7	7	8	8
	534	101	1	N	6	10	10	9	8	6	6	6	6	6		
	70356	105	N	N	8	12	10	9	7	6	7	8	8	8		
	81652	89	1	N	8	11	11	12	7	6	6	6	5	5	7	6
	49509	89	N	N	7	13	12	11	8	8	8	8	10	10	12	
	529	92	N	N	8	12	13	10	8	6	6	6	6	6	7	7
	533	92	N	1	7	16	14	13	11	8	8	7	7	7	8	8
	539	71	1	1	6	12	12	11	8	11	11	9	10	9	8	
4 N.V. Controls	49468	96	N	N	N	N	N	1	N	3	5	7	6	6		
	54815	95	N	N	N	N	N	N	N	3	2	5	7	7	8	
	536	99	N	1	N	1	N	N	N	5	5	8	8	7		
	49480	100	1	1	1	1	1	1	1	9	10	10	8	8		
	542	95	N	N	N	1	N	1	N	6	9	10	12	11		
	70319	101	N	N	N	N	N	N	N	5	5	6	7	7		
	78429	88	N	N	N	N	N	1	N	4	7	9	9	8		
	70348	75	N	N	1	1	1	1	N	5	4	4	3	2		
	54812	71	N	1	N	1	N	1	1	3	5	6	8	7		
	25983	70	N	1	1	1	1	1	N	5	5	8	8	8		

.. signifies Not done

NV ,, Not vaccinated

I.D. ,, Intradermally

TA ,, Typical Abortion

N ,, Negative

C ,, Contaminated

Nl signifies Normal

GPD ,, Guinea pigs died

L ,, Living

P ,, Premature

The agglutination titres are expressed by tube numbers e.g. 1 = 1/10, 2 = 1/20, 3 = 1/40, etc.

2

OF S.19 VACCINE ADMINISTERED BY DIFFERENT ROUTES

History of Pregnancy					Examination at Parturition					
						Cotyledons		Foetal Stomach		Colostrum	
Service to infection (days)	Infection to Parturition days	Duration of pregnancy (days)	Parturition	Fate of calf	Blood serum titre	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.
159	82	241	TA	D	10	+	+ 5/5	C	N 0/5	N	+ 5/5
181	79	260	TA	D	8	+	+ 5/5	+	+ 5/5	N	+ 5/5
166	87	253	P	L	5	+	+ 5/5	..	..	N	+ 4/5
166	116	282	Nl	L	1	N	N 0/5	..	..	N	N 0/5
155	121	276	Nl	L	5	N	N 0/5	..	..	N	N 0/5
141	87	228	TA	D	9	+	GPD	C	N 0/3	+	+ 2/5
140	108	248	TA	D	6	+	+ 5/5	+	+ 4/4	N	+ 4/4
139	141	280	Nl	L	2	C	N 0/5	..	..	C	N 0/4
135	138	273	Nl	L	7	N	N 0/5	..	..	N	N 0/5
151	119	270	Nl	L	1	N	N 0/5	..	..	N	N 0/5
161	54	215	TA	D	8	N	..	+	+ 5/5	+	+ 5/5
161	119	280	Nl	L	6	N	N 0/5	..	..	N	N 0/5
155	129	284	Nl	L	3	N	N 0/4	..	..	N	N 0/5
161	70	231	TA	D	8	+	+ 5/5	N	+ 5/5	N	N 0/5
162	86	248	P	L	8	+	+ 5/5	..	..	N	N 0/5
166	106	272	P	L	10	+	+ 2/5	..	..	N	N 0/5
162	119	281	Nl	L	7	N	N 0/5	..	..	N	N 0/5
133	147	280	Nl	L	7	..	N 0/3	..	..	C	N 0/3
134	142	276	Nl	L	7	C	+ 4/4	..	..	C	+ 3/5
143	136	279	Dystokia	D	3	C	N 0/5	..	GPD	..	N 0/5
159	106	265	TA	D	8	+	+ 5/5	+	+ 4/4	N	N 0/5
154	109	263	P	L	8	+	+ 5/5	..	..	+	+ 5/5
161	122	283	Nl	L	8	N	N 0/5	..	..	N	N 0/5
161	116	277	Nl	L	6	N	N 0/5	..	..	N	N 0/6
165	70	235	TA	D	8	..	+ 4/4	N	+ 5/5	N	N 0/5
149	126	275	Nl	L	6	N	N 0/5	..	..	N	N 0/5
149	96	245	P	L	9	+	+ 4/4	..	..	N	+ 5/5
152	123	275	Nl	L	7	N	N 0/5	..	..	N	N 0/5
152	133	285	Nl	L	7	N	N 0/4	..	..	N	N 0/5
131	105	236	TA	D	10	+	+ 1/4	+	+ 3/5	N	N 0/5
156	65	221	TA	D	8	N	+ 3/3	N	N 0/3	C	+ 2/3
155	111	266	TA	D	7	C	+ 3/3	C	N 0/3	N	+ 2/2
159	66	225	TA	D	8	+	..	+	..	N	+ 3/3
160	67	227	TA	D	14	+	..	+	..	C	+ 3/3
155	70	225	TA	D	14	C	+ 3/3	+	..	+	..
161	58	219	TA	D	5	+	..	+	..	N	+ 1/3
148	76	224	TA	D	11	+	..	+	..	N	+ 3/3
135	55	190	TA	D	4	+	..	+	..	N	+ 2/3
131	84	215	TA	D	6	C	+ 3/3	N	N 0/3	N	+ 1/3
130	78	208	TA	D	7	+	..	+	..	N	+ 3/3

N 0/5 signifies Five pigs negative

S.C. ,, Subcutaneously

D ,, Dead

+ ,, Positive

earlier findings. A small local necrotic lesion, which healed rapidly, was produced at the inoculation site.

Large dose (5.0 ml.) subcutaneously. In the majority of cases, the local and systemic reactions following inoculation were typical of those usually produced by this particular dose. These reactions which are much larger than those associated with the small dosage have been described previously (Edwards et al., 1946 ; McDiarmid, 1949, 1950). Two heifers showed no apparent reactions.

Immunity Produced

The results obtained following the application of the infective dose are shown in detail in Table 2 and summarised in Table 3. Figure 1 depicts the mean agglutination titres in the different groups throughout the course of the experiment.

Subcutaneous group (small dose). By this method of vaccination, protection against the test dose was afforded to five heifers out of ten. It is interesting to note that one animal (95350), eventually proved to be immune, had failed to respond to vaccination in a satisfactory manner when judged by the agglutination titre. On no occasion subsequent to vaccination did the titre of this particular heifer rise above 1 in 40. The five animals infected in this group produced four dead calves and infection was detected in four of the cotyledon samples, two of the samples from foetal stomachs and all the colostrum samples. Nineteen per cent. of the post partum milk samples were infected.

Intradermal group. A similar protection rate was obtained as in the previous group. Four cotyledons, two foetal stomach samples and two colostrum samples were found infected from five infected heifers, but only two dead calves in this group could be attributed to *Br. abortus* infection. The milk was comparatively free from infection, compared with the other three groups, six out of ninety samples containing *Br. abortus* (6.7 per cent).

Subcutaneous group (large dose). The protective rate was again of the same order as in the previous two groups, namely five out of ten animals. The cotyledons from all five infected animals contained *Br. abortus* and in addition, the stomach contents of three out of three dead calves were infected. Only two colostrum samples were positive. The incidence of excretion of *Br. abortus* in the milk (23 per cent), closely corresponded to the figure obtained in the group vaccinated with the smaller dose by the same route.

With one exception, all strains of *Br. abortus* isolated from the experimental heifers were CO_2 sensitive and produced persisting infection in guinea pigs similar to that produced by strain 544. In heifer 25992 the only strain isolated at parturition was aerobic, highly sensitive to thionin blue and of reduced virulence for guinea pigs. These findings support the belief that S.19 was responsible for the abortion in this instance.

TABLE 3

SUMMARY OF RESULTS

Group	*Method of vaccination*	*Mean duration of pregnancy*	*Number of Heifers infected*	*Number of living calves*	*Excretion of* Br. abortus *in the milk*	
					Number of samples examined	*Number of samples positive*
1 10 heifers	0.2ml. S.19 vaccine subcutaneously	261	5	6	100	19
2 10 heifers	0.2ml. S.19 vaccine intradermally	265	5	7	90	6
3 10 heifers	5.0ml. S.19 vaccine subcutaneously	264	5	7	100	23
4 10 heifers	Non-vaccinated controls	222	10	0	100	33

Controls. (*Non-vaccinated group*). The infective dose used in this experiment appeared to be of a satisfactory nature since all ten animals showed evidence of infection at the time of parturition. *Br. abortus* was isolated from the cotyledons in every case, from the foetal stomachs in seven of the calves and from the colostrum of every heifer. All ten calves were born dead. Thirty-three per cent of post-partum milk samples were infected.

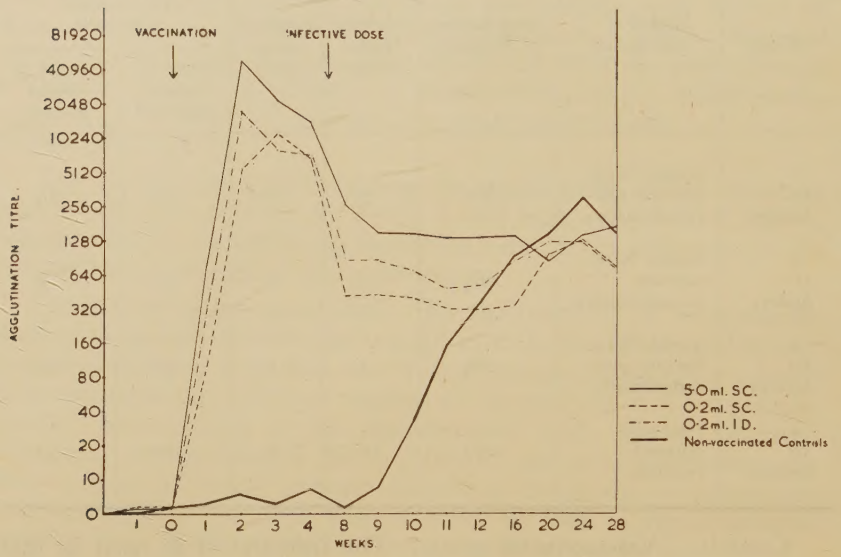
DISCUSSION

Previous findings, (McDiarmid, 1950) have confirmed the theory of Campbell and Rodwell (1945) that a small dose of S.19 vaccine given intradermally would produce a satisfactory degree of immunity to brucellosis. It was not possible to assemble sufficient animals for this preliminary experiment to prove whether the route *per se* was wholly responsible for the successful response to this dose of antigen, or whether the same number of organisms given subcutaneously would have produced a similar result. In the present experiment the immunity produced by 0.2 ml. S.19 injected intradermally was shown to be equal to that obtained by the same quantity of vaccine given subcutaneously, but in both groups and in the group vaccinated with 5.0 ml. the level of immunity was poor in comparison with previous results since only five of the ten vaccinated animals were protected compared with the usual eight.

Various suggestions can be advanced to account for this result. At the commencement of the experiment pregnant heifers were used,

Fig. 1

MEAN AGGLUTINATION TITRES.



owing to a scarcity of non-pregnant animals and a desire to obtain an answer to this question at an early date because of further work contemplated to explore the duration of immunity following vaccination. It is feasible that the pregnant heifer does not respond so well to the introduction of *Br. abortus* antigen as does the non-pregnant animal and that consequently the antibody level is correspondingly lower. Another factor which must be considered is the shorter interval between the time of vaccination and the application of the challenge dose ; it could be deduced that this had to some extent influenced the final outcome of this experiment. The efficiency of this batch of vaccine was carefully checked by viable plate counts and by the inoculation of guinea pigs at Weybridge prior to issue and was apparently quite satisfactory with regard to the number of viable organisms present and the subsequent protection afforded to the guinea pigs. Its protective power in cattle was, of course, not known prior to this experiment. If it was purely a question of viable organisms present in the vaccine, a better protection rate could perhaps have been expected in the group inoculated with 5.0 ml. compared with the smaller dose groups since this dose contains approximately twenty five times the number of organisms. The infective dose was carefully checked by viable count and by inoculation of experimental animals and was, in fact, lower than the estimated amount. The possibility that immunity had been overwhelmed by an excessive test dose can therefore be excluded.

It is extremely difficult to decide which factor or factors were responsible for the results obtained. It can only be stressed that the two main deviations in this experiment from our previous technique were the use of animals already pregnant at vaccination and a diminished interval between the immunisation of the heifers and the application of the test dose ; it must, however, be borne in mind that the amount of evidence available concerning the application of this particular test dose to vaccinated cattle is still somewhat limited. The recovery of only one strain of *Br. abortus* which possessed the character of reduced virulence usually associated with S.19, again confirms previous findings, (McDiarmid, 1951) that vaccination of pregnant cattle can be carried out without undue risk of abortion due to vaccination. This is of considerable importance when dealing with an abortion storm in a non-vaccinated herd.

CONCLUSIONS

A small dose (0.2 ml.) of S.19 *Br. abortus* vaccine administered subcutaneously was shown to confer an immunity to heifers in no way inferior to that produced by the same dose intradermally or by a larger dose (5.0 ml.) by the subcutaneous route. The protective power of the vaccine did not appear to be as effective as in previous experiments. Various factors are discussed which may have been responsible for this phenomenon.

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